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(54) Title: PATCHED GENES AND THEIR USE

(57) Abstract

Invertebrate and vertebrate *patched* genes are provided, including the mouse and human *patched* genes, as well as methods for isolation of related genes, where the genes may be of different species or in the same family. Having the ability to regulate the expression of the *patched* gene, allows for the elucidation of embryonic development, cellular regulation associated with signal transduction by the *patched* gene, the identification of agonist and antagonist to signal transduction, identification of ligands for binding to *patched*, isolation of the ligands, and assaying for levels of transcription and expression of the *patched* gene.

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PATCHED GENES AND THEIR USE

5

INTRODUCTION

Technical Field

The field of this invention concerns segment polarity genes and their uses.

10 Background

Segment polarity genes were discovered in flies as mutations which change the pattern of structures of the body segments. Mutations in the genes cause animals to develop the changed patterns on the surfaces of body segments, the changes affecting the pattern along the head to tail axis. For example, mutations in the gene

15 *patched* cause each body segment to develop without the normal structures in the center of each segment. In their stead is a mirror image of the pattern normally found in the anterior segment. Thus cells in the center of the segment make the wrong structures, and point them in the wrong direction with reference to the over all head-to-tail polarity of the animal. About sixteen genes in the class are known.

20 The encoded proteins include kinases, transcription factors, a cell junction protein, two secreted proteins called wingless (WG) and hedgehog (HH), a single transmembrane protein called *patched* (PTC), and some novel proteins not related to any known protein. All of these proteins are believed to work together in signaling pathways that inform cells about their neighbors in order to set cell fates and

25 polarities.

Many of the segment polarity proteins of *Drosophila* and other invertebrates are closely related to vertebrate proteins, implying that the molecular mechanisms involved are ancient. Among the vertebrate proteins related to the fly genes are En-1 and -2, which act in vertebrate brain development and WNT-1, which is also involved in brain development, but was first found as the oncogene implicated in many cases of mouse breast cancer. In flies, the *patched* gene is transcribed into RNA in a complex and dynamic pattern in embryos, including fine transverse stripes in each body segment primordium. The encoded protein is predicted to contain many transmembrane domains. It has no significant similarity to any other known protein. Other proteins having large numbers of transmembrane domains include a variety of membrane receptors, channels through membranes and transporters through membranes.

The hedgehog (HH) protein of flies has been shown to have at least three vertebrate relatives: *Sonic hedgehog* (*Shh*); *Indian hedgehog*, and *Desert hedgehog*. The *Shh* is expressed in a group of cells at the posterior of each developing limb bud. This is exactly the same group of cells found to have an important role in signaling polarity to the developing limb. The signal appears to be graded, with cells close to the posterior source of the signal forming posterior digits and other limb structures and cells farther from the signal source forming more anterior structures. It has been known for many years that transplantation of the signaling cells, a region of the limb bud known as the "zone of polarizing activity (ZPA)" has dramatic effects on limb patterning. Implanting a second ZPA anterior to the limb bud causes a limb to develop with posterior features replacing the anterior ones (in essence little fingers instead of thumbs). *Shh* has been found to be the long sought ZPA signal. Cultured cells making *Shh* protein (SHH), when implanted into the anterior limb bud region, have the same effect as an implanted ZPA. This establishes that *Shh* is clearly a critical trigger of posterior limb development.

The factor in the ZPA has been thought for some time to be related to another important developmental signal that polarizes the developing spinal cord. The notochord, a rod of mesoderm that runs along the dorsal side of early vertebrate embryos, is a signal source that polarizes the neural tube along the dorsal-ventral axis. The signal causes the part of the neural tube nearest to the notochord to form

floor plate, a morphologically distinct part of the neural tube. The floor plate, in turn, sends out signals to the more dorsal parts of the neural tube to further determine cell fates. The ZPA was reported to have the same signaling effect as the notochord when transplanted to be adjacent to the neural tube, suggesting the ZPA makes the same signal as the notochord. In keeping with this view, *Shh* was found to be produced by notochord cells and floor plate cells. Tests of extra expression of *Shh* in mice led to the finding of extra expression of floor plate genes in cells which would not normally turn them on. Therefore *Shh* appears to be a component of the signal from notochord to floor plate and from floor plate to more dorsal parts of the neural tube. Besides limb and neural tubes, vertebrate hedgehog genes are also expressed in many other tissues including, but not limited to the peripheral nervous system, brain, lung, liver, kidney, tooth primordia, genitalia, and hindgut and foregut endoderm.

PTC has been proposed as a receptor for HH protein based on genetic experiments in flies. A model for the relationship is that PTC acts through a largely unknown pathway to inactivate both its own transcription and the transcription of the *wingless* segment polarity gene. This model proposes that HH protein, secreted from adjacent cells, binds to the PTC receptor, inactivates it, and thereby prevents PTC from turning off its own transcription or that of *wingless*. A number of experiments have shown coordinate events between PTC and HH.

Relevant Literature

Descriptions of *patched*, by itself or its role with *hedgehog* may be found in Hooper and Scott, Cell 59, 751-765 (1989); Nakano et al., Nature, 341, 508-513 (1989) (both of which also describes the sequence for *Drosophila patched*) Simcox et al., Development 107, 715-722 (1989); Hidalgo and Ingham, Development, 110, 291-301 (1990); Phillips et al., Development, 110, 105-114 (1990); Sampedro and Guerrero, Nature 353, 187-190 (1991); Ingham et al., Nature 353, 184-187 (1991); and Taylor et al., Mechanisms of Development 42, 89-96 (1993). Discussions of the role of *hedgehog* include Riddle et al., Cell 75, 1401-1416 (1993); Echelard et al., Cell 75, 1417-1430 (1993); Krauss et al., Cell 75, 1431-1444 (1993); Tabata and Kornberg, Cell 76, 89-102 (1994); Heemskerk & DiNardo, Cell 76, 449-460 (1994); Relink et al., Cell 76, 761-775 (1994); and a short review article by

Ingham, Current Biology 4, 347-350 (1994). The sequence for the *Drosophila* 5' non-coding region was reported to the GenBank, accession number M28418, referred to in Hooper and Scott (1989), *supra*. See also, Forbes, et al., Development 1993 Supplement 115-124.

5

SUMMARY OF THE INVENTION

Methods for isolating *patched* genes, particularly mammalian *patched* genes, including the mouse and human *patched* genes, as well as invertebrate *patched* genes and sequences, are provided. The methods include identification of *patched* genes from other species, as well as members of the same family of proteins. The subject genes provide methods for producing the patched protein, where the genes and proteins may be used as probes for research, diagnosis, binding of *hedgehog* protein for its isolation and purification, gene therapy, as well as other utilities.

15

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph having a restriction map of about 10kbp of the 5' region upstream from the initiation codon of *Drosophila patched* gene and bar graphs of constructs of truncated portions of the 5' region joined to β -galactosidase, where the constructs are introduced into fly cell lines for the production of embryos. The expression of β -gal in the embryos is indicated in the right-hand table during early and late development of the embryo. The greater the number of '+'s, the more intense the staining.

25

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Methods are provided for identifying members of the *patched* (*ptc*) gene family from invertebrate and vertebrate, *e.g.* mammalian, species, as well as the entire cDNA sequence of the mouse and human *patched* gene. Also, sequences for invertebrate *patched* genes are provided. The *patched* gene encodes a transmembrane protein having a large number of transmembrane sequences.

30

In identifying the mouse and human *patched* genes, primers were employed to move through the evolutionary tree from the known *Drosophila ptc* sequence. Two primers are employed from the *Drosophila* sequence with appropriate

restriction enzyme linkers to amplify portions of genomic DNA of a related invertebrate, such as mosquito. The sequences are selected from regions which are not likely to diverge over evolutionary time and are of low degeneracy.

Conveniently, the regions are the N-terminal proximal sequence, generally within
5 the first 1.5kb, usually within the first 1kb, of the coding portion of the cDNA, conveniently in the first hydrophilic loop of the protein. Employing the polymerase chain reaction (PCR) with the primers, a band can be obtained from mosquito genomic DNA. The band may then be amplified and used in turn as a probe. One may use this probe to probe a cDNA library from an organism in a different branch
10 of the evolutionary tree, such as a butterfly. By screening the library and identifying sequences which hybridize to the probe, a portion of the butterfly *patched* gene may be obtained. One or more of the resulting clones may then be used to rescreen the library to obtain an extended sequence, up to and including the entire coding region, as well as the non-coding 5'- and 3'-sequences. As
15 appropriate, one may sequence all or a portion of the resulting cDNA coding sequence.

One may then screen a genomic or cDNA library of a species higher in the evolutionary scale with appropriate probes from one or both of the prior sequences. Of particular interest is screening a genomic library, of a distantly related
20 invertebrate, *e.g.* beetle, where one may use a combination of the sequences obtained from the previous two species, in this case, the *Drosophila* and the butterfly. By appropriate techniques, one may identify specific clones which bind to the probes, which may then be screened for cross hybridization with each of the probes individually. The resulting fragments may then be amplified, *e.g.* by
25 subcloning.

By having all or parts of the 4 different *patched* genes, in the presently illustrated example, *Drosophila* (fly), mosquito, butterfly and beetle, one can now compare the *patched* genes for conserved sequences. Cells from an appropriate mammalian limb bud or other cells expressing *patched*, such as notochord, neural
30 tube, gut, lung buds, or other tissue, particularly fetal tissue, may be employed for screening. Alternatively, adult tissue which produces *patched* may be employed for screening. Based on the consensus sequence available from the 4 other species, one

can develop probes where at each site at least 2 of the sequences have the same nucleotide and where the site varies that each species has a unique nucleotide, inosine may be used, which binds to all 4 nucleotides.

Either PCR may be employed using primers or, if desired, a genomic library
5 from an appropriate source may be probed. With PCR, one may use a cDNA library or use reverse transcriptase-PCR (RT-PCR), where mRNA is available from the tissue. Usually, where fetal tissue is employed, one will employ tissue from the first or second trimester, preferably the latter half of the first trimester or the second trimester, depending upon the particular host. The age and source of tissue will
10 depend to a significant degree on the ability to surgically isolate the tissue based on its size, the level of expression of *patched* in the cells of the tissue, the accessibility of the tissue, the number of cells expressing *patched* and the like. The amount of tissue available should be large enough so as to provide for a sufficient amount of mRNA to be usefully transcribed and amplified. With mouse tissue, limb bud of
15 from about 10 to 15 dpc (days post conception) may be employed.

In the primers, the complementary binding sequence will usually be at least 14 nucleotides, preferably at least about 17 nucleotides and usually not more than about 30 nucleotides. The primers may also include a restriction enzyme sequence for isolation and cloning. With RT-PCR, the mRNA may be enriched in accordance
20 with known ways, reverse transcribed, followed by amplification with the appropriate primers. (Procedures employed for molecular cloning may be found in Molecular Cloning: A Laboratory Manual, Sambrook et al., eds., Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, 1988). Particularly, the primers may conveniently come from the N-terminal proximal sequence or other conserved
25 region, such as those sequences where at least five amino acids are conserved out of eight amino acids in three of the four sequences. This is illustrated by the sequences (SEQ ID NO:11) IITPLDCFWEG, (SEQ ID NO:12) LIVGG, and (SEQ ID NO:13) PFFWEQY. Resulting PCR products of expected size are subcloned and may be sequenced if desired.

30 The cloned PCR fragment may then be used as a probe to screen a cDNA library of mammalian tissue cells expressing *patched*, where hybridizing clones may be isolated under appropriate conditions of stringency. Again, the cDNA library

should come from tissue which expresses *patched*, which tissue will come within the limitations previously described. Clones which hybridize may be subcloned and rescreened. The hybridizing subclones may then be isolated and sequenced or may be further analyzed by employing RNA blots and *in situ* hybridizations in whole and
5 sectioned embryos. Conveniently, a fragment of from about 0.5 to 1 kbp of the N-terminal coding region may be employed for the Northern blot.

The mammalian gene may be sequenced and as described above, conserved regions identified and used as primers for investigating other species. The N-terminal proximal region, the C-terminal region or an intermediate region may be
10 employed for the sequences, where the sequences will be selected having minimum degeneracy and the desired level of conservation over the probe sequence.

The DNA sequence encoding PTC may be cDNA or genomic DNA or fragment thereof, particularly complete exons from the genomic DNA, may be isolated as the sequence substantially free of wild-type sequence from the
15 chromosome, may be a 50 kbp fragment or smaller fragment, may be joined to heterologous or foreign DNA, which may be a single nucleotide, oligonucleotide of up to 50 bp, which may be a restriction site or other identifying DNA for use as a primer, probe or the like, or a nucleic acid of greater than 50 bp, where the nucleic acid may be a portion of a cloning or expression vector, comprise the regulatory
20 regions of an expression cassette, or the like. The DNA may be isolated, purified being substantially free of proteins and other nucleic acids, be in solution, or the like.

The subject gene may be employed for producing all or portions of the *patched* protein. The subject gene or fragment thereof, generally a fragment of at
25 least 12 bp, usually at least 18 bp, may be introduced into an appropriate vector for extrachromosomal maintenance or for integration into the host. Fragments will usually be immediately joined at the 5' and/or 3' terminus to a nucleotide or sequence not found in the natural or wild-type gene, or joined to a label other than a nucleic acid sequence. For expression, an expression cassette may be employed,
30 providing for a transcriptional and translational initiation region, which may be inducible or constitutive, the coding region under the transcriptional control of the transcriptional initiation region, and a transcriptional and translational termination

region. Various transcriptional initiation regions may be employed which are functional in the expression host. The peptide may be expressed in prokaryotes or eukaryotes in accordance with conventional ways, depending upon the purpose for expression. For large production of the protein, a unicellular organism or cells of a higher organism, *e.g.* eukaryotes such as vertebrates, particularly mammals, may be used as the expression host, such as *E. coli*, *B. subtilis*, *S. cerevisiae*, and the like. In many situations, it may be desirable to express the *patched* gene in a mammalian host, whereby the *patched* gene will be transported to the cellular membrane for various studies. The protein has two parts which provide for a total of six transmembrane regions, with a total of six extracellular loops, three for each part. The character of the protein has similarity to a transporter protein. The protein has two conserved glycosylation signal triads.

The subject nucleic acid sequences may be modified for a number of purposes, particularly where they will be used intracellularly, for example, by being joined to a nucleic acid cleaving agent, *e.g.* a chelated metal ion, such as iron or chromium for cleavage of the gene; as an antisense sequence; or the like. Modifications may include replacing oxygen of the phosphate esters with sulfur or nitrogen, replacing the phosphate with phosphoramidate, etc.

With the availability of the protein in large amounts by employing an expression host, the protein may be isolated and purified in accordance with conventional ways. A lysate may be prepared of the expression host and the lysate purified using HPLC, exclusion chromatography, gel electrophoresis, affinity chromatography, or other purification technique. The purified protein will generally be at least about 80% pure, preferably at least about 90% pure, and may be up to 100% pure. By pure is intended free of other proteins, as well as cellular debris.

The polypeptide may be used for the production of antibodies, where short fragments provide for antibodies specific for the particular polypeptide, whereas larger fragments or the entire gene allow for the production of antibodies over the surface of the polypeptide or protein, where the protein may be in its natural conformation.

Antibodies may be prepared in accordance with conventional ways, where the expressed polypeptide or protein may be used as an immunogen, by itself or

conjugated to known immunogenic carriers, *e.g.* KLH, pre-S HBsAg, other viral or eukaryotic proteins, or the like. Various adjuvants may be employed, with a series of injections, as appropriate. For monoclonal antibodies, after one or more booster injections, the spleen may be isolated, the splenocytes immortalized, and then
5 screened for high affinity antibody binding. The immortalized cells, *e.g.* hybridomas, producing the desired antibodies may then be expanded. For further description, see *Monoclonal Antibodies: A Laboratory Manual*, Harlow and Lane eds., Cold Spring Harbor Laboratories, Cold Spring Harbor, New York, 1988. If desired, the mRNA encoding the heavy and light chains may be isolated and
10 mutigenized by cloning in *E. coli*, and the heavy and light chains may be mixed to further enhance the affinity of the antibody. The antibodies may find use in diagnostic assays for detection of the presence of the PTC protein on the surface of cells or to inhibit the transduction of signal by the PTC protein ligand by competing for the binding site.

15 The mouse *patched* gene (SEQ ID NO:09) encodes a protein (SEQ ID NO:10) which has about 38% identical amino acids to fly PTC (SEQ ID NO:6) over about 1,200 amino acids. This amount of conservation is dispersed through much of the protein excepting the C-terminal region. The mouse protein also has a 50 amino acid insert relative to the fly protein. The human *patched* gene (SEQ ID
20 NO:18) contains an open reading frame of about 1450 amino acids (SEQ ID NO:19) that is about 96% identical (98 % similar) to mouse *ptc* (SEQ ID NO:09). The human *patched* gene (SEQ ID NO:18), including coding and non-coding sequences, is about 89% identical to the mouse *patched* gene (SEQ ID NO:09).

The butterfly PTC homolog (SEQ ID NO:4) is 1,300 amino acids long and
25 overall has a 50% amino acid identity (72% similarity) to fly PTC (SEQ ID NO:6). With the exception of a divergent C-terminus, this homology is evenly spread across the coding sequence. A 267bp exon from the beetle *patched* gene encodes an 89 amino acid protein fragment which was found to be 44% and 51% identical to the corresponding regions of fly and butterfly PTC respectively.

30 The mouse *ptc* message is about 8 kb long and the message is present in low levels as early as 7 dpc, the abundance increasing by 11 and 15 dpc. Northern blot indicates a clear decrease in the amount of message at 17 dpc. In the adult, PTC

RNA is present in high amounts in the brain and lung, as well as in moderate amounts in the kidney and liver. Weak signals are detected in heart, spleen, skeletal muscle and testes.

- In mouse embryos, *ptc* mRNA is present at 7 dpc, using *in situ* hybridization. *ptc* is present at high levels along the neural axis of 8.5 dpc embryos. By 11.5 dpc, *ptc* can be detected in developing lung buds and gut, consistent with its Northern profile. In addition, the gene is present at high levels in the ventricular zone of the central nervous system as well as in the zona limitans of the prosencephalon. *ptc* is also strongly transcribed in the perichondrium condensing cartilage of 11.5 and 13.5 dpc limb buds, as well as in the ventral portion of the somites, a region which is prospective sclerotome and eventually forms bone in the vertebral column. PTC is present in a wide range of tissues from endodermal, mesodermal, as well as ectodermal origin, evidencing the fundamental role in many aspects of embryonic development, including the condensation of cartilage, the patterning of limbs, the differentiation of lung tissue, and the generation of neurons.

- The *patched* nucleic acid may be used for isolating the gene from various mammalian sources of interest, particularly primate, more particularly human, or from domestic animals, both pet and farm, *e.g.* lagomorpha, rodentiae, porcine, bovine, feline, canine, ovine, equine, etc. By using probes, particularly labeled probes of DNA sequences, of the *patched* gene, one may be able to isolate mRNA or genomic DNA, which may be then used for identifying mutations, particularly associated with genetic diseases, such as spina bifida, limb defects, lung defects, problems with tooth development, liver and kidney development, peripheral nervous system development, and other sites where a *patched* gene is involved in regulation.
- The subject probes can also be used for identifying the level of expression in cells associated with the testis to determine the relationship with the level of expression and sperm production.

- The gene or fragments thereof may be used as probes for identifying the 5' non-coding region comprising the transcriptional initiation region, particularly the enhancer regulating the transcription of *patched*. By probing a genomic library, particularly with a probe comprising the 5' coding region, one can obtain fragments comprising the 5' non-coding region. If necessary, one may walk the fragment to

obtain further 5' sequence to ensure that one has at least a functional portion of the enhancer. It is found that the enhancer is proximal to the 5' coding region, a portion being in the transcribed sequence and downstream from the promoter sequences. The transcriptional initiation region may be used for many purposes,
5 studying embryonic development, providing for regulated expression of *patched* protein or other protein of interest during embryonic development or thereafter, and in gene therapy.

The gene may also be used for gene therapy, by transfection of the normal gene into embryonic stem cells or into mature cells. A wide variety of viral vectors
10 can be employed for transfection and stable integration of the gene into the genome of the cells. Alternatively, micro-injection may be employed, fusion, or the like for introduction of genes into a suitable host cell. See, for example, Dhawan *et al.*, Science 254, 1509-1512 (1991) and Smith *et al.*, Molecular and Cellular Biology (1990) 3268-3271.

15 By providing for the production of large amounts of PTC protein, one can use the protein for identifying ligands which bind to the PTC protein. Particularly, one may produce the protein in cells and employ the polysomes in columns for isolating ligands for the PTC protein. One may incorporate the PTC protein into liposomes by combining the protein with appropriate lipid surfactants, *e.g.*
20 phospholipids, cholesterol, etc., and sonicate the mixture of the PTC protein and the surfactants in an aqueous medium. With one or more established ligands, *e.g.* *hedgehog*, one may use the PTC protein to screen for antagonists which inhibit the binding of the ligand. In this way, drugs may be identified which can prevent the transduction of signals by the PTC protein in normal or abnormal cells.

25 The PTC protein, particularly binding fragments thereof, the gene encoding the protein, or fragments thereof, particularly fragments of at least about 18 nucleotides, frequently of at least about 30 nucleotides and up to the entire gene, more particularly sequences associated with the hydrophilic loops, may be employed in a wide variety of assays. In these situations, the particular molecules will
30 normally be joined to another molecule, serving as a label, where the label can directly or indirectly provide a detectable signal. Various labels include radioisotopes, fluorescers, chemilumescers, enzymes, specific binding molecules,

particles, *e.g.* magnetic particles, and the like. Specific binding molecules include pairs, such as biotin and streptavidin, digoxin and antidigoxin etc. For the specific binding members, the complementary member would normally be labeled with a molecule which provides for detection, in accordance with known procedures. The

5 assays may be used for detecting the presence of molecules which bind to the *patched* gene or PTC protein, in isolating molecules which bind to the *patched* gene, for measuring the amount of *patched*, either as the protein or the message, for identifying molecules which may serve as agonists or antagonists, or the like.

Various formats may be used in the assays. For example, mammalian or

10 invertebrate cells may be designed where the cells respond when an agonist binds to PTC in the membrane of the cell. An expression cassette may be introduced into the cell, where the transcriptional initiation region of *patched* is joined to a marker gene, such as β -galactosidase, for which a substrate forming a blue dye is available. A 1.5kb fragment that responds to PTC signaling has been identified and shown to

15 regulate expression of a heterologous gene during embryonic development. When an agonist binds to the PTC protein, the cell will turn blue. By employing a competition between an agonist and a compound of interest, absence of blue color formation will indicate the presence of an antagonist. These assays are well known in the literature. Instead of cells, one may use the protein in a membrane

20 environment and determine binding affinities of compounds. The PTC may be bound to a surface and a labeled ligand for PTC employed. A number of labels have been indicated previously. The candidate compound is added with the labeled ligand in an appropriate buffered medium to the surface bound PTC. After an incubation to ensure that binding has occurred, the surface may be washed free of

25 any non-specifically bound components of the assay medium, particularly any non-specifically bound labeled ligand, and any label bound to the surface determined. Where the label is an enzyme, substrate producing a detectable product may be used. The label may be detected and measured. By using standards, the binding affinity of the candidate compound may be determined.

30 The availability of the gene and the protein allows for investigation of the development of the fetus and the role *patched* and other molecules play in such development. By employing antisense sequences of the *patched* gene, where the

sequences may be introduced in cells in culture, or a vector providing for transcription of the antisense of the *patched* gene introduced into the cells, one can investigate the role the PTC protein plays in the cellular development. By providing for the PTC protein or fragment thereof in a soluble form which can compete with
5 the normal cellular PTC protein for ligand, one can inhibit the binding of ligands to the cellular PTC protein to see the effect of variation in concentration of ligands for the PTC protein on the cellular development of the host. Antibodies against PTC can also be used to block function, since PTC is exposed on the cell surface.

The subject gene may also be used for preparing transgenic laboratory
10 animals, which may serve to investigate embryonic development and the role the PTC protein plays in such development. By providing for variation in the expression of the PTC protein, employing different transcriptional initiation regions which may be constitutive or inducible, one can determine the developmental effect of the differences in PTC protein levels. Alternatively, one can use the DNA to
15 knock out the PTC protein in embryonic stem cells, so as to produce hosts with only a single functional *patched* gene or where the host lacks a functional *patched* gene. By employing homologous recombination, one can introduce a *patched* gene, which is differentially regulated, for example, is expressed to the development of the fetus, but not in the adult. One may also provide for expression of the *patched* gene in
20 cells or tissues where it is not normally expressed or at abnormal times of development. One may provide for mis-expression or failure of expression in certain tissue to mimic a human disease. Thus, mouse models of spina bifida or abnormal motor neuron differentiation in the developing spinal cord are made available. In addition, by providing expression of PTC protein in cells in which it is
25 otherwise not normally produced, one can induce changes in cell behavior upon binding of ligand to the PTC protein.

Areas of investigation may include the development of cancer treatments. The *wingless* gene, whose transcription is regulated in flies by PTC, is closely related to a mammalian oncogene, *Wnt-1*, a key factor in many cases of mouse
30 breast cancer. Other Wnt family members, which are secreted signaling proteins, are implicated in many aspects of development. In flies, the signaling factor *decapentaplegic*, a member of the TGF-beta family of signaling proteins, known to

affect growth and development in mammals, is also controlled by PTC. Since members of both the TGF-beta and Wnt families are expressed in mice in places close to overlapping with *patched*, the common regulation provides an opportunity in treating cancer. Also, for repair and regeneration, proliferation competent cells making PTC protein can find use to promote regeneration and healing for damaged tissue, which tissue may be regenerated by transfecting cells of damaged tissue with the *ptc* gene and its normal transcription initiation region or a modified transcription initiation region. For example, PTC may be useful to stimulate growth of new teeth by engineering cells of the gums or other tissues where PTC protein was during an earlier developmental stage or is expressed.

Since Northern blot analysis indicates that *ptc* is present at high levels in adult lung tissue, the regulation of *ptc* expression or binding to its natural ligand may serve to inhibit proliferation of cancerous lung cells. The availability of the gene encoding PTC and the expression of the gene allows for the development of agonists and antagonists. In addition, PTC is central to the ability of neurons to differentiate early in development. The availability of the gene allows for the introduction of PTC into host diseased tissue, stimulating the fetal program of division and/or differentiation. This could be done in conjunction with other genes which provide for the ligands which regulate PTC activity or by providing for agonists other than the natural ligand.

The availability of the coding region for various *ptc* genes from various species, allows for the isolation of the 5' non-coding region comprising the promoter and enhancer associated with the *ptc* genes, so as to provide transcriptional and post-transcriptional regulation of the *ptc* gene or other genes, which allow for regulation of genes in relation to the regulation of the *ptc* gene. Since the *ptc* gene is autoregulated, activation of the *ptc* gene will result in activation of transcription of a gene under the transcriptional control of the transcriptional initiation region of the *ptc* gene. The transcriptional initiation region may be obtained from any host species and introduced into a heterologous host species, where such initiation region is functional to the desired degree in the foreign host. For example, a fragment of from about 1.5 kb upstream from the initiation codon, up to about 10kb, preferably up to about 5 kb may be used to provide for transcriptional initiation regulated by

the PTC protein, particularly the *Drosophila* 5'-non-coding region (GenBank accession no. M28418).

The following examples are offered by illustration not by way of limitation.

5

EXPERIMENTAL

Methods and Materials

I. PCR on Mosquito (*Anopheles gambiae*) Genomic DNA:

PCR primers were based on amino acid stretches of fly PTC that were not likely to diverge over evolutionary time and were of low degeneracy. Two such primers (P2R1 (SEQ ID NO:14): GGACGAATTCAARGTNCA^YCARYTNTGG, P4R1: (SEQ ID NO:15) GGACGAATTCCYTCCCARAARCANTC, (the underlined sequences are Eco RI linkers) amplified an appropriately sized band from mosquito genomic DNA using the PCR. The program conditions were as follows:

15 94 °C 4 min.; 72 °C Add Taq;
 [49 °C 30 sec.; 72 °C 90 sec.; 94 °C 15 sec] 3 times
 [94 °C 15 sec.; 50 °C 30 sec.; 72 °C 90 sec] 35 times
 72 °C 10 min; 4 °C hold

This band was subcloned into the EcoRV site of pBluescript II and sequenced using
20 the USB Sequence kit.

II. Screen of a Butterfly cDNA Library with Mosquito PCR Product

Using the mosquito PCR product (SEQ ID NO:7) as a probe, a 3 day embryonic *Precis coenia* λgt10 cDNA library (generously provided by Sean
25 Carroll) was screened. Filters were hybridized at 65 °C overnight in a solution containing 5xSSC, 10% dextran sulfate, 5x Denhardt's, 200 µg/ml sonicated salmon sperm DNA, and 0.5% SDS. Filters were washed in 0.1X SSC, 0.1% SDS at room temperature several times to remove nonspecific hybridization. Of the
30 100,000 plaques initially screened, 2 overlapping clones, L1 and L2, were isolated, which corresponded to the N terminus of butterfly PTC. Using L2 as a probe, the library filters were rescreened and 3 additional clones (L5, L7, L8) were isolated which encompassed the remainder of the *ptc* coding sequence. The full length

sequence of butterfly *ptc* (SEQ ID NO:3) was determined by ABI automated sequencing.

III. Screen of a *Tribolium* (beetle) Genomic Library with Mosquito PCR Product and 900 bp Fragment from the Butterfly Clone

5 A λ gem11 genomic library from *Tribolium castaneum* (gift of Rob Dennell) was probed with a mixture of the mosquito PCR (SEQ ID NO:7) product and BstXI/EcoRI fragment of L2. Filters were hybridized at 55 °C overnight and washed as above. Of the 75,000 plaques screened, 14 clones were identified and the
10 SacI fragment of T8 (SEQ ID NO:1), which crosshybridized with the mosquito and butterfly probes, was subcloned into pBluescript.

IV. PCR on Mouse cDNA Using Degenerate Primers Derived from Regions Conserved in the Four Insect Homologues

15 Two degenerate PCR primers (P4REV: (SEQ ID NO:16) GGACGAATTCYTNGANTGYTTYTGGGA; P22: (SEQ ID NO:17) CATACCAGCCAAGCTTGTCIGGCCARTGCAT) were designed based on a comparison of PTC amino acid sequences from fly (*Drosophila melanogaster*) (SEQ ID NO:6), mosquito (*Anopheles gambiae*) (SEQ ID NO:8), butterfly (*Precis coenia*) (SEQ ID NO:4), and beetle (*Tribolium castaneum*) (SEQ ID NO:2).
20 I represents inosine, which can form base pairs with all four nucleotides. P22 was used to reverse transcribe RNA from 12.5 dpc mouse limb bud (gift from David Kingsley) for 90 min at 37 °C. PCR using P4REV (SEQ ID NO:17) and P22 (SEQ ID NO:18) was then performed on 1 μ l of the resultant cDNA under the following
25 conditions:

94 °C 4 min.; 72 °C Add Taq;

[94 °C 15 sec.; 50 °C 30 sec.; 72 °C 90 sec.] 35 times

72 °C 10 min.; 4 °C hold

PCR products of the expected size were subcloned into the TA vector (Invitrogen)
30 and sequenced with the Sequenase Version 2.0 DNA Sequencing Kit (U.S.B.).

Using the cloned mouse PCR fragment as a probe, 300,000 plaques of a mouse 8.5 dpc λ gt10 cDNA library (a gift from Brigid H gan) were screened at

65 °C as above and washed in 2x SSC, 0.1% SDS at room temperature. 7 clones were isolated, and three (M2 M4, and M8) were subcloned into pBluescript II. 200,000 plaques of this library were rescreened using first, a 1.1 kb EcoRI fragment from M2 to identify 6 clones (M9-M16) and secondly a mixed probe containing the most N terminal (XhoI fragment from M2) and most C terminal sequences (BamHI/BglII fragment from M9) to isolate 5 clones (M17-M21). M9, M10, M14, and M17-21 were subcloned into the EcoRI site of pBluescript II (Stratagene).

V. RNA Blots and in situ Hybridizations in Whole and Sectioned Mouse Embryos
10 Northern:

A mouse embryonic Northern blot and an adult multiple tissue Northern blot (obtained from Clontech) were probed with a 900 bp EcoRI fragment from an N terminal coding region of mouse *ptc*. Hybridization was performed at 65 °C in 5x SSPE, 10x Denhardt's, 100 µg/ml sonicated salmon sperm DNA, and 2% SDS. 15 After several short room temperature washes in 2x SSC, 0.05% SDS, the blots were washed at high stringency in 0.1X SSC, 0.1% SDS at 50C.

In situ hybridization of sections:

7.75, 8.5, 11.5, and 13.5 dpc mouse embryos were dissected in PBS and frozen in Tissue-Tek medium at -80 °C. 12-16 µm frozen sections were cut, 20 collected onto VectaBond (Vector Laboratories) coated slides, and dried for 30-60 minutes at room temperature. After a 10 minute fixation in 4% paraformaldehyde in PBS, the slides were washed 3 times for 3 minutes in PBS, acetylated for 10 minutes in 0.25% acetic anhydride in triethanolamine, and washed three more times for 5 minutes in PBS. Prehybridization (50% formamide, 5X SSC, 250 µg/ml yeast 25 tRNA, 500 µg/ml sonicated salmon sperm DNA, and 5x Denhardt's) was carried out for 6 hours at room temperature in 50% formamide/5x SSC humidified chambers. The probe, which consisted of 1 kb from the N-terminus of *ptc*, was added at a concentration of 200-1000 ng/ml into the same solution used for prehybridization, and then denatured for five minutes at 80 °C. Approximately 75 30 µl of probe were added to each slide and covered with Parafilm. The slides were incubated overnight at 65 °C in the same humidified chamber used previously. The following day, the probe was washed successively in 5X SSC (5 minutes, 65 °C),

0.2X SSC (1 hour, 65 °C), and 0.2X SSC (10 minutes, room temperature). After five minutes in buffer B1 (0.1M maleic acid, 0.15 M NaCl, pH 7.5), the slides were blocked for 1 hour at room temperature in 1% blocking reagent (Boehringer-Mannheim) in buffer B1, and then incubated for 4 hours in buffer B1 containing the DIG-AP conjugated antibody (Boehringer-Mannheim) at a 1:5000 dilution. Excess antibody was removed during two 15 minute washes in buffer B1, followed by five minutes in buffer B3 (100 mM Tris, 100mM NaCl, 5mM MgCl₂, pH 9.5). The antibody was detected by adding an alkaline phosphatase substrate (350 µl 75 mg/ml X-phosphate in DMF, 450 µl 50 mg/ml NBT in 70% DMF in 100 mls of buffer B3) and allowing the reaction to proceed over-night in the dark. After a brief rinse in 10 mM Tris, 1mM EDTA, pH 8.0, the slides were mounted with Aquamount (Lerner Laboratories).

VI. Drosophila 5-transcriptional initiation region β-gal constructs.

A series of constructs were designed that link different regions of the *ptc* promoter from *Drosophila* to a *LacZ* reporter gene in order to study the cis regulation of the *ptc* expression pattern. See Fig. 1. A 10.8kb BamHI/BspM1 fragment comprising the 5'-non-coding region of the mRNA at its 3'-terminus was obtained and truncated by restriction enzyme digestion as shown in Fig. 1. These expression cassettes were introduced into *Drosophila* lines using a P-element vector (Thummel et al., Gene 74, 445-456 (1988), which were injected into embryos, providing flies which could be grown to produce embryos. (See Spradling and Rubin, Science (1982) 218, 341-347 for a description of the procedure.) The vector used a pUC8 background into which was introduced the white gene to provide for yellow eyes, portions of the P-element for integration, and the constructs were inserted into a polylinker upstream from the *LacZ* gene. The resulting embryos were stained using antibodies to LacZ protein conjugated to HRP and the embryos developed with OPD dye to identify the expression of the *LacZ* gene. The staining pattern is described in Fig. 1, indicating whether there was staining during the early and late development of the embryo.

VII. Isolation of a Mouse *ptc* Gene

Homologues of fly PTC (SEQ ID NO:6) were isolated from three insects: mosquito, butterfly and beetle, using either PCR or low stringency library screens. PCR primers to six amino acid stretches of PTC of low mutatability and degeneracy were designed. One primer pair, P2 and P4, amplified an homologous fragment of *ptc* from mosquito genomic DNA that corresponded to the first hydrophilic loop of the protein. The 345bp PCR product (SEQ ID NO:7) was subcloned and sequenced and when aligned to fly PTC, showed 67% amino acid identity.

The cloned mosquito fragment was used to screen a butterfly λ GT 10 cDNA library. Of 100,000 plaques screened, five overlapping clones were isolated and used to obtain the full length coding sequence. The butterfly PTC homologue (SEQ ID NO:4) is 1,311 amino acids long and overall has 50% amino acid identity (72% similarity) to fly PTC. With the exception of a divergent C-terminus, this homology is evenly spread across the coding sequence. The mosquito PCR clone (SEQ ID NO:7) and a corresponding fragment of butterfly cDNA were used to screen a beetle λ gem11 genomic library. Of the plaques screened, 14 clones were identified. A fragment of one clone (T8), which hybridized with the original probes, was subcloned and sequenced. This 3kb piece contains an 89 amino acid exon (SEQ ID NO:2) which is 44% and 51% identical to the corresponding regions of fly and butterfly PTC respectively.

Using an alignment of the four insect homologues in the first hydrophilic loop of the PTC, two PCR primers were designed to a five and six amino acid stretch which were identical and of low degeneracy. These primers were used to isolate the mouse homologue using RT-PCR on embryonic limb bud RNA. An appropriately sized band was amplified and upon cloning and sequencing, it was found to encode a protein 65% identical to fly PTC. Using the cloned PCR product and subsequently, fragments of mouse *ptc* cDNA, a mouse embryonic λ cDNA library was screened. From about 300,000 plaques, 17 clones were identified and of these, 7 form overlapping cDNA's which comprise most of the protein-coding sequence (SEQ ID NO:9).

VIIa. Developmental and Tissue Distribution of Mouse PTC RNA

In both the embryonic and adult Northern blots, the *ptc* probe detects a single 8kb message. Further exposure does not reveal any additional minor bands.

Developmentally, *ptc* mRNA is present in low levels as early as 7 dpc and becomes quite abundant by 11 and 15 dpc. While the gene is still present at 17 dpc, the

- 5 Northern blot indicates a clear decrease in the amount of message at this stage. In the adult, *ptc* RNA is present in high amounts in the brain and lung, as well as in moderate amounts in the kidney and liver. Weak signals are detected in heart, spleen, skeletal muscle, and testes.

10 VIIb. *In situ* Hybridization of Mouse PTC in Whole and Section Embryos

Northern analysis indicates that *ptc* mRNA is present at 7 dpc, while there is no detectable signal in sections from 7.75 dpc embryos. This discrepancy is explained by the low level of transcription. In contrast, *ptc* is present at high levels along the neural axis of 8.5 dpc embryos. By 11.5 dpc, *ptc* can be detected in the

- 15 developing lung buds and gut, consistent with its adult Northern profile. In addition, the gene is present at high levels in the ventricular zone of the central nervous system, as well as in the zona limitans of the prosencephalon. *ptc* is also strongly transcribed in the condensing cartilage of 11.5 and 13.5 dpc limb buds, as well as in the ventral portion of the somites, a region which is prospective
- 20 sclerotome and eventually forms bone in the vertebral column. *ptc* is present in a wide range of tissues from endodermal, mesodermal and ectodermal origin supporting its fundamental role in embryonic development.

VIII. Isolation of the Human *ptc* Gene

- 25 To isolate human *ptc* (hptc), 2×10^5 plaques from a human lung cDNA library (HL3022a, Clontech) were screened with a 1kbp mouse *ptc* fragment, M2-2. Filters were hybridized overnight at reduced stringency (60 °C in 5X SSC, 10% dextran sulfate, 5X Denhardt's, 0.2 mg/ml sonicated salmon sperm DNA, and 0.5% SDS). Two positive plaques (H1 and H2) were isolated, the inserts cloned into
- 30 pBluescript, and upon sequencing, both contained sequence highly similar to the mouse *ptc* homolog. To isolate the 5' end, an additional 6×10^5 plaques were screened in duplicate with M2-3 EcoR I and M2-3 Xho I (containing 5' untranslated

sequence of mouse *ptc* probes. Ten plaques were purified and of these, 6 inserts were subcloned into pBluescript. To obtain the full coding sequence, H2 was fully and H14, H20, and H21 were partially sequenced. The 5.1kbp of human *ptc* sequence (SEQ ID NO:18) contains an open reading frame of 1447 amino acids
 5 (SEQ ID NO:19) that is 96% identical and 98% similar to mouse *ptc*. The 5' and 3' untranslated sequences of human *ptc* (SEQ ID NO:18) are also highly similar to mouse *ptc* (SEQ ID NO:09) suggesting conserved regulatory sequence.

IX. Comparison of Mouse, Human, Fly and Butterfly Sequences

10 The deduced mouse PTC protein sequence (SEQ ID NO:10) has about 38% identical amino acids to fly PTC over about 1,200 amino acids. This amount of conservation is dispersed through much of the protein excepting the C-terminal region. The mouse protein also has a 50 amino acid insert relative to the fly
 15 of *hedgehog* between fly and mouse, one concludes that *ptc* functions similarly in the two organisms. A comparison of the amino acid sequences of mouse (mptc) (SEQ ID NO:10), human (hptc) (SEQ ID NO:19), butterfly (bptc)(SEQ ID NO:4) and drosophila (ptc) (SEQ ID NO:6) is shown in Table 1.

TABLE 1

20	alignment of human, mouse, fly, and butterfly PTC homologs	
	alignment of human, mouse, fly, and butterfly ptc homologs	
25	HPTC	MASAGNAAEPQDR--GGGGSGCIGAPGRPAGGGRRRRRTGGGLRRRAAPDRDYLHRPSYCDA
	MPTC	MASAGNAA-----GALGRQAGGGRRRRRTGGGPHRA-APDRDYLHRPSYCDA
	PTC	M-----DRDSLPRVPDTHGD--VVDE-----KLFSDL-----YI-RTSWVDA
30	BPTC	MVAPDSEAPSNPRITAAHESPCATEA-----RHSADL-----YI-RTSWVDA
		* . . . * . * . *
	HPTC	AFALEQISKGKATGRKAPLWLRKAFQRLLFKLGCYIQKNCGKFLVVGLLIFGAFVGLKA
	MPTC	AFALEQISKGKATGRKAPLWLRKAFQRLLFKLGCYIQKNCGKFLVVGLLIFGAFVGLKA
35	PTC	QVALDQIDKKGKARGSRTAIYLRVSFQSHLETGSSVQKHAGKVLFAVLVLSTFCVGLKS
	BPTC	ALALSELEKGNIEGGRTSLWIRAWLQEQFLGCFLOGDAGKVLFAVLVLSTFCVGLKS
		** .. ** . * * . * . * . * . * . * . * . * . * . *
	HPTC	ANLETNVEELWVEVGGRVSRELNYTRQKIGEEAMFNPQLMIQTPKEEGANVLTTEALLQH
40	MPTC	ANLETNVEELWVEVGGRVSRELNYTRQKIGEEAMFNPQLMIQTPKEEGANVLTTEALLQH
	PTC	AQIHSKVHQLWIEGGRLEAEALAYTQKTIGEDESATHQLLIQTTHDPNASVLHPQALLAH
	BPTC	AQIHTRVDQLWVQEGGRLEAEALKYTAQALGEADSSTHQLVIQTAKDPDVSLLHPGALLEH
		* * . * . . . * . * . * . * . * . * . * . * . * . *
	HPTC	LDSALQASRVHVYMYNRQWKLEHLCKYSGELITET-GYMDQIIEYLYPCLIIITPLDCFWE

WO 96/11260

MPTC LDSALQASRVHVYMYNRQWKLEHLCKYKSGELITET-GYMDQIIEYLYPCLIIITPLDCFEW
 PTC LEVLVKATAVKVHLYDTEWGLRDMCNMPTSPSFEGIIYIEQILRHLPCSIITPLDCFEW
 BPTC LKVVHAATRVTVHMYDIEWRLKDLCSYSPIDFEGYHHIESIIDNVIPCAIITPLDCFEW
 * * * * *

5 HPTC GAKLQSGTAYLLGKPPLR-----WTNFDPLEFLEELK-----KINYQVDSWEEMLNKAEV
 MPTC GAKLQSGTAYLLGKPPLR-----WTNFDPLEFLEELK-----KINYQVDSWEEMLNKAEV
 PTC GSQLL-GPESAVVPIGLNQRLLWTTLNPAVMQYMKQKMSSEEKISFDFETVEQYMKRAAI
 BPTC GSKLL-GPDYPIYVPHLKHKLQWTHLNPLEVVEVK-KL---KFQFPLSTIEAYMKRAGI
 * * * * *

10 HPTC GHGYMDRPCLNPAADPCPATAPNKNSTKPLDMALVLNGGCHGLSRKYMHWQEELIVGGTV
 MPTC GHGYMDRPCLNPAADPCPATAPNKNSTKPLDVALVLNGGCGQLSRKYMHWQEELIVGGTV
 PTC GSGYMEKPCLNPLNPNCPDTAPNKNSTQPPDVGAILSGGCYGYAAKHMHWPEELIVGGRK
 BPTC TSAYMKKPCLDPTDPHCPATAPNKKSGHIPDVAAELSHGCYGFAAAYMHWPEQLIVGGAT
 * * * * *

15 HPTC KNSTGKLVSAHALQTMFQMTPKOMYEHFKGYEYVSHINWNEDKAAAILAWQRTYVEVV
 MPTC KNATGKLVSAHALQTMFQMTPKOMYEHFRGYDYVSHINWNEDRAAAAILAWQRTYVEVV
 PTC RNRSGHLRKAQALQSVVQLMTEKEMYDQWQDNKVVHHLGWTQEKAAEVNLAQWQNFESREV
 BPTC RNSTALRSARALQTVVQLMGEREMYEWADHYKVHQIGWNQEKAAAVLDAWQRFKFAEV
 * * * * *

20 HPTC HQSVAQNSTQK-----VLSEFTTTTLDLILKSFSDSVIRVASGYLLMLAYACLTMLRW-DC
 MPTC HQSVAPNSTQK-----VLPFTTTTLDLILKSFSDSVIRVASGYLLMLAYACLTMLRW-DC
 PTC EQLLRKQSRIATNYDIYVSSAALDDILAKFSHPSALSIVIGVAVTVLYAFCTLLRWRDP
 BPTC RKI-TTSGSVSSAYSFPFSTSTLNDILGKFSEVSLKNIILGYMFMLIYVAVTTLIQWRDP
 * * * * *

25 HPTC SKSQGAVGLAGVLLVALSVAAGLGLCSLIGISFNAATTQVLPFLALGVGVDDVFLLAHAF
 MPTC SKSQGAVGLAGVLLVALSVAAGLGLCSLIGISFNAATTQVLPFLALGVGVDDVFLLAHAF
 PTC VRGQSSVGAVGVLMLCFSTAAGLGLSALLGIVFNAASTQVVPFLALGLGVDDHIFMLTAAY
 BPTC IRSQAGVGIAGVLLSITVAAGLGFALLGIPFNASTQIVPFLALGLGVDDMFLLTHTY
 * * * * *

30 HPTC SETGQNKRIPFEDRTGECLKRTGASVALTSISNVTAFMAALIPIPALRAFSLQAAVVVV
 MPTC SETGQNKRIPFEDRTGECLKRTGASVALTSISNVTAFMAALIPIPALRAFSLQAAVVVV
 PTC AESN-----RREQTKLILKKVGPSILFSACSTAGSFFAAAFIPVPALKVFCLOAAIVMC
 BPTC VEQAGD--VPREERTGLVLKKSGLSVLLASLCNVMAFLAAALLPIPAFRVFCLOAAIILL
 * * * * *

35 HPTC FNFAMVLLIFPAILSMDLRREDRRLDIFCCFTSPCVSRVIOVEPQAYTDTHDNTRYSP
 MPTC FNFAMVLLIFPAILSMDLRREDRRLDIFCCFTSPCVSRVIOVEPQAYTEPHSNTRYSP
 PTC SNLAAALLVFPAMISLDLRRRTAGRADIFCCCF-PVWKEQPKVAPPVLPNNNNGR----
 BPTC FNLGSILLVFPAMISLDLRRRSARADLLCCLM-P---ESP-----LPKKKIPER----
 * * * * *

40 HPTC PPYSSHSFAHETQITMQSTVQLRTEYDPHTHVYYTTAEPRSEISVQPVTVTQDT LSCQSP
 MPTC PPYTSHSFAHETHITMQSTVQLRTEYDPHTHVYYTTAEPRSEISVQPVTVTQDNLSQSP
 PTC -----GARHPKSCNNRVPLPAQNPLLEQRA
 BPTC -----AKTRKNDKTHRID-TTROPLDPDVS
 * * * * *

45 HPTC ESTSSTRDLLSQFSDSSLHCLEPPCTKWTLSFAEKHYAPFLKPKAKVVVIFLFLGLLG
 MPTC ESTSSTRDLLSQFSDSSLHCLEPPCTKWTLSFAEKHYAPFLKPKAKVVVIFLFLGLLG
 PTC DIPGSS-----HSLASF-----SLATFAFQHYTPFLMRSWVKFLTVMGFLAALI
 BPTC ENVTKT-----CCL-SV-----SLTKWAKNQYAPFIMRPAVKVTSMLALIAVIL
 * * * * *

50 HPTC VSLYGTTRVRDGLDLTDIVPRETREYDFIAAQFKYFSFYNNYIVTQKA-DYPNIOHLLYD
 MPTC VSLYGTTRVRDGLDLTDIVPRETREYDFIAAQFKYFSFYNNYIVTQKA-DYPNIOHLLYD
 PTC SSLYASTRLQDGLDIIIDLVPKDSNEHKFLDAQTRLFYFSYMYAVTQGNFEYPTQQLLRD
 BPTC TSVWGATKVKDGLDLTDIVPENTDEHEFLSRQEKYFGFYNNYAVTQGNFEYPTQQLLYE
 * * * * *

55 HPTC
 MPTC
 PTC
 BPTC

60 HPTC
 MPTC
 PTC
 BPTC

65

The identity of ten other clones recovered from the mouse library is not determined. These cDNAs cross-hybridize with mouse *ptc* sequence, while differing as to their restriction maps. These genes encode a family of proteins related to the
5 *patched* protein. Alignment of the human and mouse nucleotide sequences, which includes coding and noncoding sequence, reveals 89% identity.

In accordance with the subject invention, mammalian *patched* genes, including the mouse and human genes, are provided which allow for high level production of
10 the *patched* protein, which can serve many purposes. The *patched* protein may be used in a screening for agonists and antagonists, for isolation of its ligand, particularly *hedgehog*, more particularly *Sonic hedgehog*, and for assaying for the transcription of the mRNA *ptc*. The protein or fragments thereof may be used to produce antibodies specific for the protein or specific epitopes of the protein. In
15 addition, the gene may be employed for investigating embryonic development, by screening fetal tissue, preparing transgenic animals to serve as models, and the like.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were
20 specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention
25 that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY
- (ii) TITLE OF INVENTION: Patched Genes and their Use
- (iii) NUMBER OF SEQUENCES: 19
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Flehr, Hohbach, Test, Albritton & Herbert
 - (B) STREET: Four Embarcadero Center, Suite 3400
 - (C) CITY: San Francisco
 - (D) STATE: CA
 - (E) COUNTRY: US
 - (F) ZIP: 94111
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: PCT/US95/
 - (B) FILING DATE: 06-OCT-1995
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Rowland, Bertram I
 - (B) REGISTRATION NUMBER: 20015
 - (C) REFERENCE/DOCKET NUMBER: a60190-1
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 415-781-1989
 - (B) TELEFAX: 415-398-3249

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 736 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AACNNC NNTN NATGGCACCC CCNCCCAACC TTTNNNCCNN NTAANCAAAA NNCCCCNTTT

60

NATACCCCT NTAAANTTT TCCACCNNNC NNAAANNCCN CTGNANACNA NGNAAANCCN 120
 TTTTNAACC CCCCCACCC GGAATTCCNA NTNNCCNCCC CCAAATTACA ACTCCAGNCC 180
 AAAATTNANA NAATTGGTCC TAACCTAACC NATNGTTGTT ACGGTTTCCC CCCCCAAATA 240
 CATGCACTGG CCCGAACACT TGATCGTTGC CGTTCCAATA AGAATAAATC TGGTCATATT 300
 AAACAAGCCN AAAGCTTTAC AACTGTTGT ACAATTAATG GCGAACACG AACTGTTCGA 360
 ATTCTGGTCT GGACATTACA AAGTGCACCA CATCGGATGG AACCAGGAGA AGGCCACAAC 420
 CGTACTGAAC GCCTGGCAGA AGAAGTTCGC ACAGGTTGGT GGTGGCGCA AGGAGTAGAG 480
 TGAATGGTGG TAATTTTGG TTGTTCCAGG AGGTGGATCG TCTGACGAAG AGCAAGAAGT 540
 CGTCGAATTA CATCTTCGTG ACGTTCTCCA CCGCCAATT GAACAAGATG TTGAAGGAGG 600
 CGTCGAANAC GGACGTGGTG AAGCTGGGGG TGGTGCTGGG GGTGGCGGCG GTGTACGGGT 660
 GGGTGGCCCA GTCGGGGCTG GCTGCCTTGG GAGTGCTGGT CTTNGCGNGC TNCNATTCGC 720
 CCTATAGTNA GNCGTA 736

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Xaa Pro Pro Pro Asn Tyr Asn Ser Xaa Pro Lys Xaa Xaa Xaa Leu Val
 1 5 10 15
 Leu Thr Pro Xaa Val Val Thr Val Ser Pro Pro Lys Tyr Met His Trp
 20 25 30
 Pro Glu His Leu Ile Val Ala Val Pro Ile Arg Ile Asn Leu Val Ile
 35 40 45
 Leu Asn Lys Pro Lys Ala Leu Gln Thr Val Val Gln Leu Met Gly Glu
 50 55 60
 His Glu Leu Phe Glu Phe Trp Ser Gly His Tyr Lys Val His His Ile
 65 70 75 80
 Gly Trp Asn Gln Glu Lys Ala Thr Thr Val Leu Asn Ala Trp Gln Lys
 85 90 95
 Lys Phe Ala Gln Val Gly Gly Trp Arg Lys Glu

100

105

(2) INFORMATION FOR SEQ ID N :3:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5187 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGGTCTGTCA CCCGGAGCCG GAGTCCCCGG CGGCCAGCAG CGTCCTCGCG AGCCGAGCGC	60
CCAGGCGCGC CCGGAGCCCG CGGCGGCGGC GGCAACATGG CCTCGGCTGG TAACGCCGCGC	120
GGGGCCCTGG GCAGGCAGGC CGGCGGCGGG AGGCCAGAC GGACCGGGGG ACCGCACCGC	180
GCCGCGCCGG ACCGGGACTA TCTGCACCGG CCCAGCTACT GCGACGCCGC CTTCGCTCTG	240
GAGCAGATTT CCAAGGGGAA GGCTACTGGC CGGAAAGCGC CGCTGTGGCT GAGAGCGAAG	300
TTTCAGAGAC TCTTATTTAA ACTGGGTTGT TACATTCAAA AGAACTGCGG CAAGTTTTTG	360
GTGTGGGTC TCCTCATATT TGGGGCCTTC GCTGTGGGAT TAAAGGCAGC TAATCTCGAG	420
ACCAACGTGG AGGAGCTGTG GGTGGAAGTT GGTGGACGAG TGAGTCGAGA ATTAAATTAT	480
ACCCGTCAGA AGATAGGAGA AGAGGCTATG TTTAATCCTC AACTCATGAT ACAGACTCCA	540
AAAGAAGAAG GCGCTAATGT TCTGACCACA GAGGCTCTCC TGCAACACCT GGAATCAGCA	600
CTCCAGGCCA GTCGTGTGCA CGTCTACATG TATAACAGGC AATGGAAGTT GGAACATTTG	660
TGCTACAAAT CAGGGGAACT TATCAGGAG ACAGGTTACA TGGATCAGAT AATAGAATAC	720
CTTTACCTT GCTTAATCAT TACACCTTTG GACTGCTTCT GGAAGGGGC AAAGCTACAG	780
TCCGGGACAG CATACCTCCT AGGTAAGCCT CTTTACGGT GGACAACTT TGACCCCTTG	840
GAATTCCTAG AAGAGTTAAA GAAAATAAAC TACCAAGTGG ACAGCTGGGA GGAAATGCTG	900
AATAAGCCG AAGTTGGCCA TGGGTACATG GACCGGCCTT GCCTCAACCC AGCCGACCCA	960
GATTGCCCTG CCACAGCCCC TAACAAAAAT TCAACCAAAC CTCTTGATGT GGCCCTTGTT	1020
TTGAATGGTG GATGTCAAGG TTTATCCAGG AAGTATATGC ATTGGCAGGA GGAGTTGATT	1080
GTGGGTGGTA CCGTCAAGAA TGCCACTGGA AAAGTTGTCA GCGCTCACGC CCTGCAAACC	1140
ATGTTCCAGT TAATGACTCC CAAGCAAATG TATGAACACT TCAGGGGCTA CGACTATGTC	1200
TCTCACATCA ACTGGAATGA AGACAGGGCA GCCGCCATCC TGGAGGCCTG GCAGAGGACT	1260

TACGTGGAGG TGGTTCATCA AAGTGTGCGC CCAAACCTCCA CTCAAAAGGT GCTTCCCTTC	1320
ACAACCACGA CCCTGGACGA CATCCTAAAA TCCTTCTCTG ATGTCAGTGT CATCCGAGTG	1380
GCCAGCGGCT ACCTACTGAT GCTTGCCTAT GCCTGTTTAA CCATGCTGCG CTGGGACTGC	1440
TCCAAGTCCC AGGGTGCCGT GGGGCTGGCT GGCCTCCTGT TGGTTGCGCT GTCAGTGGCT	1500
GCAGGATTGG GCCTCTGCTC CTTGATTGGC ATTTCTTTTA ATGCTGCGAC AACTCAGGTT	1560
TGCGCGTTTC TTGCTCTTGG TGTGGGTGTG GATGATGTCT TCCTCCTGGC CCATGCATTG	1620
AGTGAAACAG GACAGAATAA GAGGATTCCA TTTGAGGACA GGAAGTGGGA GTGCCTCAAG	1680
CGCACCGGAG CCAGCGTGGC CCTCACCTCC ATCAGCAATG TCACCGCCTT CTTGATGGCC	1740
GCATTGATCC CTATCCCTGC CCTGCGAGCG TTCTCCCTCC AGGCTGCTGT GGTGGTGGTA	1800
TTCAATTTTG CTATGGTTCT GCTCATTTTT CCTGCAATTC TCAGCATGGA TTTATACAGA	1860
CGTGAGGACA GAAGATTGGA TATTTTCTGC TGTTTCACAA GCCCCTGTGT CAGCAGGGTG	1920
ATTCAAGTTG AGCCACAGGC CTACACAGAG CCTCACAGTA ACACCCGGTA CAGCCCCCA	1980
CCCCATACA CCAGCCACAG CTTGCCCCAC GAAACCCATA TCACTATGCA GTCCACCGTT	2040
CAGCTCCGCA CAGAGTATGA CCCTCACAGC CACGTGTACT ACACCACCGC CGAGCCACGC	2100
TCTGAGATCT CTGTACAGCC TGTACCGTC ACCCAGGACA ACCTCAGCTG TCAGAGTCCC	2160
GAGAGCACCA GCTCTACCAG GGACCTGCTC TCCCAGTTCT CAGACTCCAG CCTCCACTGC	2220
CTCGAGCCCC CCTGCACCAA GTGGACACTC TCTTCGTTTG CAGAGAAGCA CTATGCTCCT	2280
TTCTCCTGA AACCCTAAGC CAAGGTTGTG GTAATCCTTC TTTTCTGGG CTGCTGGGG	2340
GTCAGCCTTT ATGGGACCAC CCGAGTGAGA GACGGGCTGG ACCTCACGGA CATTGTTCCC	2400
CGGGAAACCA GAGAATATGA CTTCATAGCT GCCCAGTTCA AGTACTTCTC TTTCTACAAC	2460
ATGTATATAG TCACCCAGAA AGCAGACTAC CCGAATATCC AGCACCTACT TTACGACCTT	2520
CATAAGAGTT TCAGCAATGT GAAGTATGTC ATGCTGGAGG AGAACAAGCA ACTTCCCCAA	2580
ATGTGGCTGC ACTACTTTAG AGACTGGCTT CAAGGACTTC AGGATGCATT TGACAGTGAC	2640
TGGGAAACTG GGAGGATCAT GCCAAACAAT TATAAAAATG GATCAGATGA CGGGGTCCTC	2700
GCTTACAAAC TCCTGGTGCA GACTGGCAGC CGAGACAAGC CCATCGACAT TAGTCAGTTG	2760
ACTAAACAGC GTCTGGTAGA CGCAGATGGC ATCATTATC CGAGCGCTTT CTACATCTAC	2820
CTGACCGCTT GGGTCAGCAA CGACCCTGTA GCTTACGCTG CCTCCCAGGC CAACATCOGG	2880
CCTCACCGGC CGGAGTGGGT CCATGACAAA GCCGACTACA TGCCAGAGAC CAGGCTGAGA	2940
ATCCCAGCAG CAGAGCCCAT CGAGTACGCT CAGTTCCCTT TCTACCTCAA CGGCCTACGA	3000

GACACCTCAG ACTTTGTGGA AGCCATAGAA AAAGTGAGAG TCATCTGTAA CAACTATACG	3060
AGCCTGGGAC TGTCCAGCTA CCCCAATGGC TACCCCTTCC TGTTCTGGGA GCAATACATC	3120
AGCCTGCGCC ACTGGCTGCT GCTATCCATC AGCGTGGTGC TGGCCTGCAC GTTTCTAGTG	3180
TGCGCAGTCT TCCTCCTGAA CCCCTGGACG GCCGGGATCA TTGTCATGGT CCTGGCTCTG	3240
ATGACCGTTG AGCTCTTTGG CATGATGGGC CTCATTGGGA TCAAGCTGAG TGCTGTGCCT	3300
GTGGTCATCC TGATTGCATC TGTTGGCATC GGAGTGGAGT TCACCGTCCA CGTGGCTTTG	3360
GCCTTTCTGA CAGCCATTGG GGACAAGAAC CACAGGGCTA TGCTCGCTCT GGAACACATG	3420
TTTGCTCCCG TTCTGGACGG TGCTGTGTCC ACTCTGCTGG GTGTACTGAT GCTTGCAGGG	3480
TCCGAATTTG ATTTCAATTG CAGATACTTC TTTGCCGTCC TGGCCATTCT CACCGTCTTG	3540
GGGGTTCTCA ATGGACTGGT TCTGCTGCCT GTCCTCTTAT CCTTCTTTGG ACCGTGTCCT	3600
GAGGTGTCTC CAGCCAATGG CCTAAACCGA CTGCCCCATC CTTGCGCTGA GCCGCCTCCA	3660
AGTGTGCTCC GGTTTGCCGT GCCTCCTGGT CACACGAACA ATGGGTCTGA TTCCTCCGAC	3720
TGGGAGTACA GCTCTCAGAC CACGGTGTCT GGCATCAGTG AGGAGCTCAG GCAATACGAA	3780
GCACAGCAGG GTGCCGGAGG CCCTGCCCAC CAAGTGATTG TGGAAGCCAC AGAAAACCTT	3840
GTCTTTGCCG GGTCCACTGT GGTCCATCCG GACTCCAGAC ATCAGCCTCC CTTGACCCCT	3900
CGGCAACAGC CCCACCTGGA CTCTGGCTCC TTGTCCCCTG GACGGCAAGG CCAGCAGCCT	3960
CGAAGGGATC CCCCTAGAGA AGGCTTGCGG CCACCCCCCT ACAGACCGCG CAGAGACGCT	4020
TTTGAAATTT CTAAGAAGG GCATTCTGGC CTAAGCAATA GGGACCGCTC AGGGCCCCGT	4080
GGGGCCCCGT CTCACAACCC TCGGAACCCA ACGTCCACCG CCATGGGCAG CTCTGTGCCC	4140
AGCTACTGCC AGCCCATCAC CACTGTGACG GCTTCTGCTT CGGTGACTGT TGCTGTGCAT	4200
CCCCCGCCTG GACCTGGGCG CAACCCCCGA GGGGGGCCCT GTCCAGGCTA TGAGAGCTAC	4260
CCTGAGACTG ATCACGGGGT ATTTGAGGAT CCTCATGTGC CTTTTCATGT CAGGTGTGAG	4320
AGGAGGGACT CAAAGGTGGA GGTATAGAG CTACAGGACG TGGAATGTGA GGAGAGGCCG	4380
TGGGGGAGCA GCTCCAACCT AGGGTAATTA AAATCTGAAG CAAAGAGGCC AAAGATTGGA	4440
AAGCCCCGCC CCCACCTCTT TCCAGAACTG CTTGAAGAGA ACTGCTTGGG ATTATGGGAA	4500
GGCAGTTCAT TGTTACTGTA ACTGATTGTA TTATTKRGTG AAATATTTCT ATAAATATTT	4560
AARAGGTGTA CACATGTAAT ATACATGGAA ATGCTGTACA GTCTATTTCC TGGGGCCTCT	4620
CCACTCCTGC CCCAGAGTGG GGAGACCACA GGGGCCCTTT CCCCTGTGTA CATTGGTCTC	4680
TGTGCCACAA CCAAGCTTAA CTTAGTTTAA AAAAAATCT CCCAGCATAT GTCGCTGCTG	4740

CTTAAATATT GTATAATTTA CTTGTATAAT TCTATGCAAA TATTGCTTAT GTAATAGGAT 4800
 TATTTGTAAA GGTTCCTGTT TAAAATATTT TAAATTTGCA TATCACAACC CTGTGGTAGG 4860
 ATGAATTGTT ACTGTAACT TTTGAACACG CTATGCGTGG TAATTGTTTA ACGAGCAGAC 4920
 ATGAAGAAAA CAGGTTAATC CCAGTGGCTT CTCTAGGGGT AGTTGTATAT GGTTCGCATG 4980
 GGTGGATGTG TGTGTGCATG TGACTTTCCA ATGTACTGTA TTGTGGTTTG TTGTTGTTGT 5040
 TGCTGTTGTT GTTCATTTTG GTGTTTTTGG TTGCTTTGTA TGATCTTAGC TCTGGCCTAG 5100
 GTGGGCTGGG AAGGTCCAGG TCTTTTCTG TCGTGATGCT GGTGGAAAGG TGACCCCAAT 5160
 CATCTGTCCT ATTCTCTGGG ACTATTC 5187

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1311 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Val Ala Pro Asp Ser Glu Ala Pro Ser Asn Pro Arg Ile Thr Ala
 1 5 10 15
 Ala His Glu Ser Pro Cys Ala Thr Glu Ala Arg His Ser Ala Asp Leu
 20 25 30
 Tyr Ile Arg Thr Ser Trp Val Asp Ala Ala Leu Ala Leu Ser Glu Leu
 35 40 45
 Glu Lys Gly Asn Ile Glu Gly Gly Arg Thr Ser Leu Trp Ile Arg Ala
 50 55 60
 Trp Leu Gln Glu Gln Leu Phe Ile Leu Gly Cys Phe Leu Gln Gly Asp
 65 70 75 80
 Ala Gly Lys Val Leu Phe Val Ala Ile Leu Val Leu Ser Thr Phe Cys
 85 90 95
 Val Gly Leu Lys Ser Ala Gln Ile His Thr Arg Val Asp Gln Leu Trp
 100 105 110
 Val Gln Glu Gly Gly Arg Leu Glu Ala Glu Leu Lys Tyr Thr Ala Gln
 115 120 125
 Ala Leu Gly Glu Ala Asp Ser Ser Thr His Gln Leu Val Il Gln Thr
 130 135 140

Ala Lys Asp Pro Asp Val Ser Leu Leu His Pro Gly Ala Leu Leu Glu
 145 150 155 160
 His Leu Lys Val Val His Ala Ala Thr Arg Val Thr Val His Met Tyr
 165 170 175
 Asp Ile Glu Trp Arg Leu Lys Asp Leu Cys Tyr Ser Pro Ser Ile Pro
 180 185 190
 Asp Phe Glu Gly Tyr His His Ile Glu Ser Ile Ile Asp Asn Val Ile
 195 200 205
 Pro Cys Ala Ile Ile Thr Pro Leu Asp Cys Phe Trp Glu Gly Ser Lys
 210 215 220
 Leu Leu Gly Pro Asp Tyr Pro Ile Tyr Val Pro His Leu Lys His Lys
 225 230 235 240
 Leu Gln Trp Thr His Leu Asn Pro Leu Glu Val Val Glu Glu Val Lys
 245 250 255
 Lys Leu Lys Phe Gln Phe Pro Leu Ser Thr Ile Glu Ala Tyr Met Lys
 260 265 270
 Arg Ala Gly Ile Thr Ser Ala Tyr Met Lys Lys Pro Cys Leu Asp Pro
 275 280 285
 Thr Asp Pro His Cys Pro Ala Thr Ala Pro Asn Lys Lys Ser Gly His
 290 295 300
 Ile Pro Asp Val Ala Ala Glu Leu Ser His Gly Cys Tyr Gly Phe Ala
 305 310 315 320
 Ala Ala Tyr Met His Trp Pro Glu Gln Leu Ile Val Gly Gly Ala Thr
 325 330 335
 Arg Asn Ser Thr Ser Ala Leu Arg Lys Ala Arg Xaa Leu Gln Thr Val
 340 345 350
 Val Gln Leu Met Gly Glu Arg Glu Met Tyr Glu Tyr Trp Ala Asp His
 355 360 365
 Tyr Lys Val His Gln Ile Gly Trp Asn Gln Glu Lys Ala Ala Ala Val
 370 375 380
 Leu Asp Ala Trp Gln Arg Lys Phe Ala Ala Glu Val Arg Lys Ile Thr
 385 390 395 400
 Thr Ser Gly Ser Val Ser Ser Ala Tyr Ser Phe Tyr Pro Phe Ser Thr
 405 410 415
 Ser Thr Leu Asn Asp Ile Leu Gly Lys Phe Ser Glu Val Ser Leu Lys
 420 425 430
 Asn Il Ile Leu Gly Tyr Met Ph Met Leu Ile Tyr Val Ala Val Thr
 435 440 445
 Leu Il Gln Trp Arg Asp Pro Ile Arg Ser Gln Ala Gly Val Gly Ile

450	455	460
Ala Gly Val Leu Leu Leu Ser Ile Thr Val Ala Ala Gly Leu Gly Phe		
465	470	475 480
Cys Ala Leu Leu Gly Ile Pro Phe Asn Ala Ser Ser Thr Gln Ile Val		
	485	490 495
Pro Phe Leu Ala Leu Gly Leu Gly Val Gln Asp Met Phe Leu Leu Thr		
	500	505 510
His Thr Tyr Val Glu Gln Ala Gly Asp Val Pro Arg Glu Glu Arg Thr		
	515	520 525
Gly Leu Val Leu Lys Lys Ser Gly Leu Ser Val Leu Leu Ala Ser Leu		
	530	535 540
Cys Asn Val Met Ala Phe Leu Ala Ala Ala Leu Leu Pro Ile Pro Ala		
	545	550 555 560
Phe Arg Val Phe Cys Leu Gln Ala Ala Ile Leu Leu Leu Phe Asn Leu		
	565	570 575
Gly Ser Ile Leu Leu Val Phe Pro Ala Met Ile Ser Leu Asp Leu Arg		
	580	585 590
Arg Arg Ser Ala Ala Arg Ala Asp Leu Leu Cys Cys Leu Met Pro Glu		
	595	600 605
Ser Pro Leu Pro Lys Lys Lys Ile Pro Glu Arg Ala Lys Thr Arg Lys		
	610	615 620
Asn Asp Lys Thr His Arg Ile Asp Thr Thr Arg Gln Pro Leu Asp Pro		
	625	630 635 640
Asp Val Ser Glu Asn Val Thr Lys Thr Cys Cys Leu Ser Val Ser Leu		
	645	650 655
Thr Lys Trp Ala Lys Asn Gln Tyr Ala Pro Phe Ile Met Arg Pro Ala		
	660	665 670
Val Lys Val Thr Ser Met Leu Ala Leu Ile Ala Val Ile Leu Thr Ser		
	675	680 685
Val Trp Gly Ala Thr Lys Val Lys Asp Gly Leu Asp Leu Thr Asp Ile		
	690	695 700
Val Pro Glu Asn Thr Asp Glu His Glu Phe Leu Ser Arg Gln Glu Lys		
	705	710 715 720
Tyr Phe Gly Phe Tyr Asn Met Tyr Ala Val Thr Gln Gly Asn Phe Glu		
	725	730 735
Tyr Pro Thr Asn Gln Lys Leu Leu Tyr Glu Tyr His Asp Gln Phe Val		
	740	745 750
Arg Il Pro Asn Ile Ile Lys Asn Asp Asn Gly Gly Leu Thr Lys Phe		
	755	760 765

Trp Leu Ser Leu Phe Arg Asp Trp Leu Leu Asp Leu Gln Val Ala Phe
 770 775 780
 Asp Lys Glu Val Ala Ser Gly Cys Ile Thr Gln Glu Tyr Trp Cys Lys
 785 790 795 800
 Asn Ala Ser Asp Glu Gly Ile Leu Ala Tyr Lys Leu Met Val Gln Thr
 805 810 815
 Gly His Val Asp Asn Pro Ile Asp Lys Ser Leu Ile Thr Ala Gly His
 820 825 830
 Arg Leu Val Asp Lys Asp Gly Ile Ile Asn Pro Lys Ala Phe Tyr Asn
 835 840 845
 Tyr Leu Ser Ala Trp Ala Thr Asn Asp Ala Leu Ala Tyr Gly Ala Ser
 850 855 860
 Gln Gly Asn Leu Lys Pro Gln Pro Gln Arg Trp Ile His Ser Pro Glu
 865 870 875 880
 Asp Val His Leu Glu Ile Lys Lys Ser Ser Pro Leu Ile Tyr Thr Gln
 885 890 895
 Leu Pro Phe Tyr Leu Ser Gly Leu Ser Asp Thr Xaa Ser Ile Lys Thr
 900 905 910
 Leu Ile Arg Ser Val Arg Asp Leu Cys Leu Lys Tyr Glu Ala Lys Gly
 915 920 925
 Leu Pro Asn Phe Pro Ser Gly Ile Pro Phe Leu Phe Trp Glu Gln Tyr
 930 935 940
 Leu Tyr Leu Arg Thr Ser Leu Leu Leu Ala Leu Ala Cys Ala Leu Ala
 945 950 955 960
 Ala Val Phe Ile Ala Val Met Val Leu Leu Leu Asn Ala Trp Ala Ala
 965 970 975
 Val Leu Val Thr Leu Ala Leu Ala Thr Leu Val Leu Gln Leu Leu Gly
 980 985 990
 Val Met Ala Leu Leu Gly Val Lys Leu Ser Ala Met Pro Ala Val Leu
 995 1000 1005
 Leu Val Leu Ala Ile Gly Arg Gly Val His Phe Thr Val His Leu Cys
 1010 1015 1020
 Leu Gly Phe Val Thr Ser Ile Gly Cys Lys Arg Arg Arg Ala Ser Leu
 1025 1030 1035 1040
 Ala Leu Glu Ser Val Leu Ala Pro Val Val His Gly Ala Leu Ala Ala
 1045 1050 1055
 Ala Leu Ala Ala Ser Met Leu Ala Ala Ser Glu Cys Gly Phe Val Ala
 1060 1065 1070
 Arg Leu Phe Leu Arg Leu Leu Leu Asp Ile Val Phe Leu Gly Leu Ile

1075	1080	1085
Asp Gly Leu Leu Phe Phe Pro Ile Val Leu Ser Ile Leu Gly Pro Ala 1090	1095	1100
Ala Glu Val Arg Pro Ile Glu His Pro Glu Arg Leu Ser Thr Pro Ser 1105	1110	1115 1120
Pro Lys Cys Ser Pro Ile His Pro Arg Lys Ser Ser Ser Ser Ser Gly 1125	1130	1135
Gly Gly Asp Lys Ser Ser Arg Thr Ser Lys Ser Ala Pro Arg Pro Cys 1140	1145	1150
Ala Pro Ser Leu Thr Thr Ile Thr Glu Glu Pro Ser Ser Trp His Ser 1155	1160	1165
Ser Ala His Ser Val Gln Ser Ser Met Gln Ser Ile Val Val Gln Pro 1170	1175	1180
Glu Val Val Val Glu Thr Thr Thr Tyr Asn Gly Ser Asp Ser Ala Ser 1185	1190	1195 1200
Gly Arg Ser Thr Pro Thr Lys Ser Ser His Gly Gly Ala Ile Thr Thr 1205	1210	1215
Thr Lys Val Thr Ala Thr Ala Asn Ile Lys Val Glu Val Val Thr Pro 1220	1225	1230
Ser Asp Arg Lys Ser Arg Arg Ser Tyr His Tyr Tyr Asp Arg Arg Arg 1235	1240	1245
Asp Arg Asp Glu Asp Arg Asp Arg Asp Arg Glu Arg Asp Arg Asp Arg 1250	1255	1260
Asp Arg Asp Arg Asp Arg Asp Arg Asp Arg Asp Arg Asp Arg Asp Arg 1265	1270	1275 1280
Glu Arg Ser Arg Glu Arg Asp Arg Arg Asp Arg Tyr Arg Asp Glu Arg 1285	1290	1295
Asp His Arg Ala Ser Pro Arg Glu Lys Arg Gln Arg Phe Trp Thr 1300	1305	1310

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4434 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGAAACAAGA GAGCGAGTGA GAGTAGGGAG AGCGTCTGTG TTGTGTGTTG AGTGTGCGCC	60
ACGCACACAG GCGCAAAACA GTGCACACAG ACGCCCGCTG GGCAAGAGAG AGTGAGAGAG	120
AGAAACAGCG GCGCGCGCTC GCCTAATGAA GTTGTGGGCC TGGCTGGCGT GCCGCATCCA	180
CGAGATACAG ATACATCTCT CATGGACCGC GACAGCCTCC CACGCGTTCC GGACACACAC	240
GGCGATGTGG TCGATGAGAA ATTATTCTCG GATCTTTACA TACGCACCAG CTGGGTGGAC	300
GCCCAAGTGG CGCTCGATCA GATAGATAAG GGCAAAGCGC GTGGCAGCCG CACGGCGATC	360
TATCTGCGAT CAGTATTCCA GTCCACCTC GAAACCCTCG GCAGCTCCGT GCAAAAGCAC	420
GCGGGCAAGG TGCTATTCTG GGCTATCCTG GTGCTGAGCA CCTTCTGCGT CGGCCTGAAG	480
AGCGCCCAGA TCCACTCCAA GGTGCACCAG CTGTGGATCC AGGAGGGCGG CCGGCTGGAG	540
GCGGAACTGG CCTACACACA GAAGACGATC GCGGAGGACG AGTCGGCCAC GCATCAGCTG	600
CTCATTGAGA CGACCCACGA CCCGAACGCC TCCGTCTGTC ATCCGCAGGC GCTGCTTGCC	660
CACCTGGAGG TCCTGGTCAA GGCCACCGCC GTCAAGGTGC ACCTCTACGA CACCGAATGG	720
GGGCTGCGCG ACATGTGCAA CATGCCGAGC ACGCCCTCCT TCGAGGGCAT CTACTACATC	780
GAGCAGATCC TGCGCCACCT CATTCTGTC TCGATCATCA CGCGCTGGA CTGTTTCTGG	840
GAGGGAAGCC AGCTGTTGGG TCCGGAATCA GCGGTCGTTA TACCAGGCCT CAACCAACGA	900
CTCCTGTGGA CCACCCTGAA TCCCGCCTCT GTGATGCAGT ATATGAAACA AAAGATGTCC	960
GAGGAAAAGA TCAGCTTCGA CTTGAGACC GTGGAGCAGT ACATGAAGCG TGCGGCCATT	1020
GGCAGTGGCT ACATGGAGAA GCCCTGCCTG AACCCACTGA ATCCCAATTG CCCGGACACG	1080
GCACCGAACA AGAACAGCAC CCAGCCGCGG GATGTGGGAG CCATCCTGTC CGGAGGCTGC	1140
TACGGTTATG CCGCGAAGCA CATGCACTGG CCGGAGGAGC TGATTGTGGG CGGACGGAAG	1200
AGGAACCGCA GCGGACACTT GAGGAAGGCC CAGGCCCTGC AGTCGGTGGT GCAGCTGATG	1260
ACCGAGAAGG AAATGTACGA CCAGTGGCAG GACAACTACA AGGTGCACCA TCTTGGATGG	1320
ACGCAGGAGA AGGCAGCGGA GGTTTTGAAC GCCTGGCAGC GCAACTTTTC GCGGGAGGTG	1380
GAACAGCTGC TACGTAAACA GTCGAGAATT GCCACCAACT ACGATATCTA CGTGTTGAGC	1440
TCGGCTGCAC TGGATGACAT CCTGGCCAAG TTCTCCCATC CCAGCGCCTT GTCCATTGTC	1500
ATCGGCGTGG CCGTCACCGT TTTGTATGCC TTTTGACCGC TCCTCCGCTG GAGGGACCCC	1560
GTCCGTGGCC AGAGCAGTGT GGGCGTGGCC GGAGTTCTGC TCATGTGCTT CAGTACCGCC	1620
GCCGGATTGG GATTGTCAGC CCTGCTCGGT ATCGTTTTCA ATGCGCTGAC CGCTGCCTAT	1680
GCGGAGAGCA ATCGGCGGGA GCAGACCAAG CTGATTCTCA AGAACGCCAG CACCCAGGTG	1740

GTTCCGTTTT TGGCCCTTGG TCTGGGCGTC GATCACATCT TCATAGTGGG ACCGAGCATC	1800
CTGTTCACTG CCTGCAGCAC CGCAGGATCC TTCTTTGCGG COGCCTTTAT TCOGGTGCCG	1860
GCTTTGAAGG TATTCTGTCT GCAGGCTGCC ATCGTAATGT GCTCCAATTT GGCAGCGGCT	1920
CTATTGGTTT TTCGGGCCAT GATTTGTTG GATCTACGGA GACGTACCGC CGGCAGGGCG	1980
GACATCTTCT GCTGCTGTTT TCCGGTGTGG AAGGAACAGC CGAAGGTGGC ACCTCCGGTG	2040
CTGCCGCTGA ACAACAACAA CGGGCGGGG GCCCGGCATC CGAAGAGCTG CAACAACAAC	2100
AGGGTGCCGC TGCCCGCCCA GAATCCTCTG CTGGAACAGA GGGCAGACAT CCCTGGGAGC	2160
AGTCACTCAC TGGCGTCCTT CTCCCTGGCA ACCTTCGCCT TTCAGCACTA CACTCCCTTC	2220
CTCATGCGCA GCTGGGTGAA GTTCCTGACC GTTATGGGT TCCTGGCGGC CCTCATATCC	2280
AGCTTGTATG CCTCCACGCG CTTTCAGGAT GGCCTGGACA TTATTGATCT GGTGCCCCAAG	2340
GACAGCAACG AGCACAAGTT CCTGGATGCT CAAACTCGGC TCTTTGGCTT CTACAGCATG	2400
TATGCGGTTA CCCAGGGCAA CTTTGAATAT CCCACCCAGC AGCAGTTGCT CAGGGACTAC	2460
CATGATTCCT TTGTGCGGGT GCCACATGTG ATCAAGAATG ATAACGGTGG ACTGCCGGAC	2520
TTCTGGCTGC TGCTCTTCAG CGAGTGGCTG GGTAACTGC AAAAGATATT CGACGAGGAA	2580
TACCGCGACG GACGGCTGAC CAAGGAGTGC TGGTTCCTAA ACGCCAGCAG CGATGCCATC	2640
CTGGCCTACA AGCTAATCGT GCAAACCGGC CATGTGGACA ACCCCGTGGA CAAGGAACTG	2700
GTGCTCACCA ATCGCCTGGT CAACAGCGAT GGCATCATCA ACCAACGCGC CTTCTACAAC	2760
TATCTGTGCG CATGGGCCAC CAACGACGTC TTCGCCTACG GAGCTTCTCA GGGCAAATTG	2820
TATCCGGAAC CGCGCCAGTA TTTTCACCAA CCAACGAGT ACGATCTTAA GATACCCAAG	2880
AGTCTGCCAT TGGTCTACGC TCAGATGCCC TTTTACCTCC ACGGACTAAC AGATACCTCG	2940
CAGATCAAGA CCCTGATAGG TCATATTGCG GACCTGAGCG TCAAGTACGA GGGCTTCGGC	3000
CTGCCCAACT ATCCATCGGG CATTCCCTTC ATCTTCTGGG AGCAGTACAT GACCCTGCGC	3060
TCCTCACTGG CCATGATCCT GGCCTGCGTG CTAATCGCG CCCTGGTGCT GGTCTCCCTG	3120
CTCCTGCTCT CGTTTGGGC CGCGTTCTC GTGATCCTCA GCGTTCGGC CTCGCTGGCC	3180
CAGATCTTTG GGGCCATGAC TCTGCTGGGC ATCAAATCTC CGGCCATTCC GGCAGTCATA	3240
CTCATCCTCA GCGTGGGCAT GATGCTGTGC TTCAATGTGC TGATATCACT GGGCTTCATG	3300
ACATCCGTTG GCAACCGACA GCGCCGCGTC CAGCTGAGCA TGCAGATGTC CCTGGGACCA	3360
CTTGTCACG GCATGCTGAC CTCGGAGTG GCCGTGTTCA TGCTCTCCAC GTCGCCCTTT	3420
GAGTTTGTGA TCCGGCACTT CTGCTGGCTT CTGCTGGTGG TCTTATGCGT TGGCGCCTGC	3480

AACAGCCTTT TGGTGTCCC CATCCTACTG AGCATGGTGG GACCGGAGGC GGAGCTGGTG	3540
CCGCTGGAGC ATCCAGACCG CATATCCACG CCCTCTCCGC TGCCCGTGCG CAGCAGCAAG	3600
AGATCGGGCA AATCCTATGT GGTGCAGGGA TCGCGATCCT CGCGAGGCAG CTGCCAGAAG	3660
TCGCATCACC ACCACCACAA AGACCTTAAT GATCCATCGC TGACGACGAT CACCGAGGAG	3720
CCGCAGTCGT GGAAGTCCAG CAACTCGTCC ATCCAGATGC CCAATGATTG GACCTACCAG	3780
CCGCGGGAAC AGCGACCGC CTCCTACGCG GCGCCGCCCC CCGCCTATCA CAAGGCCGCC	3840
GCCCAGCAGC ACCACCAGCA TCAGGGCCCG CCCACAACGC CCGCGCCTCC CTTCCCGACG	3900
GCCTATCCGC CGGAGCTGCA GAGCATCGTG GTGCAGCCGG AGGTGACGGT GGAGACGACG	3960
CACTCGGACA GCAACACCAC CAAGGTGACG GCCACGGCCA ACATCAAGGT GGAGCTGGCC	4020
ATGCCCCGCA GGGCGGTGCG CAGCTATAAC TTTACGAGTT AGCACTAGCA CTAGTTCCTG	4080
TAGCTATTAG GACGTATCTT TAGACTCTAG CCTAAGCCGT AACCCTATTT GTATCTGTAA	4140
AATCGATTG TCCAGCGGT CTGCTGAGGA TTTGTTCTC ATGGATTCTC ATGGATTCTC	4200
ATGGATGCTT AAATGGCATG GTAATTGGCA AAATATCAAT TTTGTGTCT CAAAAGATG	4260
CATTAGCTTA TGGTTTCAAG ATACATTTTT AAAGAGTCCG CCAGATATTT ATATAAAAAA	4320
AATCCAAAAT CGACGTATCC ATGAAAATTG AAAAGCTAAG CAGACCCGTA TGTATGTATA	4380
TGTGTATGCA TGTTAGTTAA TTTCCGAAG TCCGGTATTT ATAGCAGCTG CCTT	4434

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1285 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met	Asp	Arg	Asp	Ser	Leu	Pro	Arg	Val	Pro	Asp	Thr	His	Gly	Asp	Val
1				5					10					15	
Val	Asp	Glu	Lys	Leu	Phe	Ser	Asp	Leu	Tyr	Ile	Arg	Thr	Ser	Trp	Val
		20						25					30		
Asp	Ala	Gln	Val	Ala	Leu	Asp	Gln	Ile	Asp	Lys	Gly	Lys	Ala	Arg	Gly
	35						40					45			
Ser	Arg	Thr	Ala	Ile	Tyr	Leu	Arg	Ser	Val	Phe	Gln	Ser	His	Leu	Glu
	50						55					60			

Thr Leu Gly Ser Ser Val Gln Lys His Ala Gly Lys Val Leu Phe Val
 65 70 75 80
 Ala Ile Leu Val Leu Ser Thr Phe Cys Val Gly Leu Lys Ser Ala Gln
 85 90 95
 Ile His Ser Lys Val His Gln Leu Trp Ile Gln Glu Gly Gly Arg Leu
 100 105 110
 Glu Ala Glu Leu Ala Tyr Thr Gln Lys Thr Ile Gly Glu Asp Glu Ser
 115 120 125
 Ala Thr His Gln Leu Leu Ile Gln Thr Thr His Asp Pro Asn Ala Ser
 130 135 140
 Val Leu His Pro Gln Ala Leu Leu Ala His Leu Glu Val Leu Val Lys
 145 150 155 160
 Ala Thr Ala Val Lys Val His Leu Tyr Asp Thr Glu Trp Gly Leu Arg
 165 170 175
 Asp Met Cys Asn Met Pro Ser Thr Pro Ser Phe Glu Gly Ile Tyr Tyr
 180 185 190
 Ile Glu Gln Ile Leu Arg His Leu Ile Pro Cys Ser Ile Ile Thr Pro
 195 200 205
 Leu Asp Cys Phe Trp Glu Gly Ser Gln Leu Leu Gly Pro Glu Ser Ala
 210 215 220
 Val Val Ile Pro Gly Leu Asn Gln Arg Leu Leu Trp Thr Thr Leu Asn
 225 230 235 240
 Pro Ala Ser Val Met Gln Tyr Met Lys Gln Lys Met Ser Glu Glu Lys
 245 250 255
 Ile Ser Phe Asp Phe Glu Thr Val Glu Gln Tyr Met Lys Arg Ala Ala
 260 265 270
 Ile Gly Ser Gly Tyr Met Glu Lys Pro Cys Leu Asn Pro Leu Asn Pro
 275 280 285
 Asn Cys Pro Asp Thr Ala Pro Asn Lys Asn Ser Thr Gln Pro Pro Asp
 290 295 300
 Val Gly Ala Ile Leu Ser Gly Gly Cys Tyr Gly Tyr Ala Ala Lys His
 305 310 315 320
 Met His Trp Pro Glu Glu Leu Ile Val Gly Gly Arg Lys Arg Asn Arg
 325 330 335
 Ser Gly His Leu Arg Lys Ala Gln Ala Leu Gln Ser Val Val Gln Leu
 340 345 350
 Met Thr Glu Lys Glu Met Tyr Asp Gln Trp Gln Asp Asn Tyr Lys Val
 355 360 365
 His His Leu ly Trp Thr Gln Glu Lys Ala Ala Glu Val Leu Asn Ala

370	375	380
Trp Gln Arg Asn Phe Ser Arg Glu Val Glu Gln Leu Leu Arg Lys Gln 385 390 395 400		
Ser Arg Ile Ala Thr Asn Tyr Asp Ile Tyr Val Phe Ser Ser Ala Ala 405 410 415		
Leu Asp Asp Ile Leu Ala Lys Phe Ser His Pro Ser Ala Leu Ser Ile 420 425 430		
Val Ile Gly Val Ala Val Thr Val Leu Tyr Ala Phe Cys Thr Leu Leu 435 440 445		
Arg Trp Arg Asp Pro Val Arg Gly Gln Ser Ser Val Gly Val Ala Gly 450 455 460		
Val Leu Leu Met Cys Phe Ser Thr Ala Ala Gly Leu Gly Leu Ser Ala 465 470 475 480		
Leu Leu Gly Ile Val Phe Asn Ala Leu Thr Ala Ala Tyr Ala Glu Ser 485 490 495		
Asn Arg Arg Glu Gln Thr Lys Leu Ile Leu Lys Asn Ala Ser Thr Gln 500 505 510		
Val Val Pro Phe Leu Ala Leu Gly Leu Gly Val Asp His Ile Phe Ile 515 520 525		
Val Gly Pro Ser Ile Leu Phe Ser Ala Cys Ser Thr Ala Gly Ser Phe 530 535 540		
Phe Ala Ala Ala Phe Ile Pro Val Pro Ala Leu Lys Val Phe Cys Leu 545 550 555 560		
Gln Ala Ala Ile Val Met Cys Ser Asn Leu Ala Ala Ala Leu Leu Val 565 570 575		
Phe Pro Ala Met Ile Ser Leu Asp Leu Arg Arg Arg Thr Ala Gly Arg 580 585 590		
Ala Asp Ile Phe Cys Cys Cys Phe Pro Val Trp Lys Glu Gln Pro Lys 595 600 605		
Val Ala Pro Pro Val Leu Pro Leu Asn Asn Asn Asn Gly Arg Gly Ala 610 615 620		
Arg His Pro Lys Ser Cys Asn Asn Asn Arg Val Pro Leu Pro Ala Gln 625 630 635 640		
Asn Pro Leu Leu Glu Gln Arg Ala Asp Ile Pro Gly Ser Ser His Ser 645 650 655		
Leu Ala Ser Phe Ser Leu Ala Thr Phe Ala Phe Gln His Tyr Thr Pro 660 665 670		
Ph Leu Met Arg Ser Trp Val Lys Phe Leu Thr Val Met Gly Phe Leu 675 680 685		

WO 96/11260

Ala Ala Leu Ile Ser Ser Leu Tyr Ala Ser Thr Arg Leu Gln Asp Gly
 690 695 700
 Leu Asp Ile Ile Asp Leu Val Pro Lys Asp Ser Asn Glu His Lys Phe
 705 710 715 720
 Leu Asp Ala Gln Thr Arg Leu Phe Gly Phe Tyr Ser Met Tyr Ala Val
 725 730 735
 Thr Gln Gly Asn Phe Glu Tyr Pro Thr Gln Gln Gln Leu Leu Arg Asp
 740 745 750
 Tyr His Asp Ser Phe Arg Val Pro His Val Ile Lys Asn Asp Asn Gly
 755 760 765
 Gly Leu Pro Asp Phe Trp Leu Leu Leu Phe Ser Glu Trp Leu Gly Asn
 770 775 780
 Leu Gln Lys Ile Phe Asp Glu Glu Tyr Arg Asp Gly Arg Leu Thr Lys
 785 790 795 800
 Glu Cys Trp Phe Pro Asn Ala Ser Ser Asp Ala Ile Leu Ala Tyr Lys
 805 810 815
 Leu Ile Val Gln Thr Gly His Val Asp Asn Pro Val Asp Lys Glu Leu
 820 825 830
 Val Leu Thr Asn Arg Leu Val Asn Ser Asp Gly Ile Ile Asn Gln Arg
 835 840 845
 Ala Phe Tyr Asn Tyr Leu Ser Ala Trp Ala Thr Asn Asp Val Phe Ala
 850 855 860
 Tyr Gly Ala Ser Gln Gly Lys Leu Tyr Pro Glu Pro Arg Gln Tyr Phe
 865 870 875 880
 His Gln Pro Asn Glu Tyr Asp Leu Lys Ile Pro Lys Ser Leu Pro Leu
 885 890 895
 Val Tyr Ala Gln Met Pro Phe Tyr Leu His Gly Leu Thr Asp Thr Ser
 900 905 910
 Gln Ile Lys Thr Leu Ile Gly His Ile Arg Asp Leu Ser Val Lys Tyr
 915 920 925
 Glu Gly Phe Gly Leu Pro Asn Tyr Pro Ser Gly Ile Pro Phe Ile Phe
 930 935 940
 Trp Glu Gln Tyr Met Thr Leu Arg Ser Ser Leu Ala Met Ile Leu Ala
 945 950 955 960
 Cys Val Leu Leu Ala Ala Leu Val Leu Val Ser Leu Leu Leu Leu Ser
 965 970 975
 Val Trp Ala Ala Val Leu Val Ile Leu Ser Val Leu Ala Ser Leu Ala
 980 985 990
 Gln Ile Phe Gly Ala Met Thr Leu Leu Gly Ile Lys Leu Ser Ala Ile

995	1000	1005
Pro Ala Val Ile Leu Ile Leu Ser Val Gly Met Met Leu Cys Phe Asn 1010	1015	1020
Val Leu Ile Ser Leu Gly Phe Met Thr Ser Val Gly Asn Arg Gln Arg 1025	1030	1035 1040
Arg Val Gln Leu Ser Met Gln Met Ser Leu Gly Pro Leu Val His Gly 1045	1050	1055
Met Leu Thr Ser Gly Val Ala Val Phe Met Leu Ser Thr Ser Pro Phe 1060	1065	1070
Glu Phe Val Ile Arg His Phe Cys Trp Leu Leu Leu Val Val Leu Cys 1075	1080	1085
Val Gly Ala Cys Asn Ser Leu Leu Val Phe Pro Ile Leu Leu Ser Met 1090	1095	1100
Val Gly Pro Glu Ala Glu Leu Val Pro Leu Glu His Pro Asp Arg Ile 1105	1110	1115 1120
Ser Thr Pro Ser Pro Leu Pro Val Arg Ser Ser Lys Arg Ser Gly Lys 1125	1130	1135
Ser Tyr Val Val Gln Gly Ser Arg Ser Ser Arg Gly Ser Cys Gln Lys 1140	1145	1150
Ser His His His His His Lys Asp Leu Asn Asp Pro Ser Leu Thr Thr 1155	1160	1165
Ile Thr Glu Glu Pro Gln Ser Trp Lys Ser Ser Asn Ser Ser Ile Gln 1170	1175	1180
Met Pro Asn Asp Trp Thr Tyr Gln Pro Arg Glu Gln Arg Pro Ala Ser 1185	1190	1195 1200
Tyr Ala Ala Pro Pro Pro Ala Tyr His Lys Ala Ala Ala Gln Gln His 1205	1210	1215
His Gln His Gln Gly Pro Pro Thr Thr Pro Pro Pro Pro Phe Pro Thr 1220	1225	1230
Ala Tyr Pro Pro Glu Leu Gln Ser Ile Val Val Gln Pro Glu Val Thr 1235	1240	1245
Val Glu Thr Thr His Ser Asp Ser Asn Thr Thr Lys Val Thr Ala Thr 1250	1255	1260
Ala Asn Ile Lys Val Glu Leu Ala Met Pro Gly Arg Ala Val Arg Ser 1265	1270	1275 1280
Tyr Asn Phe Thr Ser 1285		

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 345 bas pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

AAGGTCCATC AGCTTTGGAT ACAGGAAGGT GGTTCGCTCG AGCATGAGCT AGCCTACACG      60
CAGAAATCGC TCGGCGAGAT GGACTCCTCC ACGCACCAGC TGCTAATCCA AACNCCCCAA      120
GATATGGACG CCTCGATACT GCACCCGAAC GCGCTACTGA CGCACCTGGA CGTGGTGAAG      180
AAAGCGATCT CGGTGACGGT GCACATGTAC GACATCACGT GGAGNCTCAA GGACATGTGC      240
TACTCGCCCA GCATACCGAG NTTGATACG CACTTTATCG AGCAGATCTT CGAGAACATC      300
ATACCGTGCG CGATCATCAC GCCGCTGGAT TGCTTTTGGG AGGGA                        345
  
```

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 115 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```

Lys Val His Gln Leu Trp Ile Gln Glu Gly Gly Ser Leu Glu His Glu
1           5           10           15
Leu Ala Tyr Thr Gln Lys Ser Leu Gly Glu Met Asp Ser Ser Thr His
20          25          30
Gln Leu Leu Ile Gln Thr Pro Lys Asp Met Asp Ala Ser Ile Leu His
35          40          45
Pro Asn Ala Leu Leu Thr His Leu Asp Val Val Lys Lys Ala Ile Ser
50          55          60
Val Thr Val His Met Tyr Asp Ile Thr Trp Xaa Leu Lys Asp Met Cys
65          70          75          80
Tyr Ser Pro Ser Ile Pro Xaa Phe Asp Thr His Phe Ile Glu Gln Ile
85          90          95
  
```

Phe Glu Asn Ile Ile Pr Cys Ala Ile Ile Thr Pro Leu Asp Cys Phe
 100 105 110

Trp Glu Gly
 115

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5187 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGGTCTGTCA CCCGGAGCCG GAGTCCCCCG CGGCCAGCAG CGTCCTCGCG AGCCGAGCGC	60
CCAGGCGCGC CCGGAGCCCG CGGCGGCGGC GGCAACATGG CCTCGGCTGG TAACGCCGCC	120
GGGGCCCTGG GCAGGCAGGC CGGCGGCGGG AGGCGCAGAC GGACCGGGGG ACCGCACCGC	180
GCCGCGCCGG ACCGGGACTA TCTGCACCGG CCCAGCTACT GCGACGCCGC CTTCGCTCTG	240
GAGCAGATTT CCAAGGGGAA GGCTACTGGC CGGAAACGC CGCTGTGGCT GAGAGCGAAG	300
TTTCAGAGAC TCTTATTTAA ACTGGGTTGT TACATTCAA AGAACTGCCG CAAGTTTTTG	360
GTTGTGGGTC TCCTCATATT TGGGGCCTTC GCTGTGGGAT TAAAGGCAGC TAATCTCGAG	420
ACCAACGTGG AGGAGCTGTG GGTGGAAGTT GGTGGACGAG TGAGTCGAGA ATTAAATTAT	480
ACCCGTCAGA AGATAGGAGA AGAGGCTATG TTTAATCCTC AACTCATGAT ACAGACTCCA	540
AAAGAAGAAG GCGCTAATGT TCTGACCACA GAGGCTCTCC TGCAACACCT GGA CTCAGCA	600
CTCCAGGCCA GTCGTGTGCA CGTCTACATG TATAACAGGC AATGGAAGTT GGAACATTG	660
TGCTACAAAT CAGGGGAACT TATCACGGAG ACAGGTTACA TGGATCAGAT AATAGAATAC	720
CTTTACCCTT GCTTAATCAT TACACCTTTG GACTGCTTCT GGAAGGGGC AAAGCTACAG	780
TCCGGGACAG CATACTCCT AGGTAAGCCT CCTTTACGCT GGACAACTT TGACCCCTTG	840
GAATTCCTAG AAGAGTTAAA GAAATAAAC TACCAAGTGG ACAGCTGGGA GGAAATGCTG	900
AATAAGCCG AAGTTGGCCA TGGGTACATG GACCGGCCTT GCCTCAACCC AGCGACCCA	960
GATTGCCCTG CCACAGCCCC TAACAAAAAT TCAACCAAAC CTCTTGATGT GGCCCTTGTT	1020
TTGAATGGTG GATGTCAAGG TTTATCCAGG AAGTATATGC ATTGGCAGGA GGAGTTGATT	1080
GTGGGTGGTA CCGTCAAGAA TGCCACTGGA AACTTGTC A GCGCTCACGC CCTGCAAACC	1140

ATGTTCCAGT TAATGACTCC CAAGCAAATG TATGAACACT TCAGGGGCTA CGACTATGTC	1200
TCTCACATCA ACTGGAATGA AGACAGGGCA GCCGCCATCC TGGAGGCCTG GCAGAGGACT	1260
TACGTGGAGG TGGTTCATCA AAGTGTCGCC CCAAACCTCCA CTCAAAAGGT GCTTCCCTTC	1320
ACAACCACGA CCCTGGACGA CATCCTAAAA TCCTTCTCTG ATGTCAGTGT CATCCGAGTG	1380
GCCAGCGGCT ACCTACTGAT GCTTGCCCTAT GCCTGTTTAA CCATGCTGCG CTGGGACTGC	1440
TCCAAGTCCC AGGGTGCCGT GGGGCTGGCT GCGTCTCTGT TGGTTGCGCT GTCAGTGGCT	1500
GCAGGATTGG GCCTCTGCTC CTTGATTGGC ATTTCTTTTA ATGCTGCGAC AACTCAGGTT	1560
TTGCCGTTTC TTGCTCTTGG TGTGGTGTG GATGATGTCT TCCTCCTGGC CCATGCATTC	1620
AGTGAAACAG GACAGAATAA GAGGATTCCA TTTGAGGACA GGAAGTGGGA GTGCCTCAAG	1680
CGCACCGGAG CCAGCGTGGC CCTCACCTCC ATCAGCAATG TCACCGCCTT CTTTCATGGC	1740
GCATTGATCC CTATCCCTGC CCTGCGAGCG TTCTCCCTCC AGGCTGCTGT GGTGGTGGTA	1800
TTCAATTTTG CTATGGTTCT GCTCATTTTT CCTGCAATTC TCAGCATGGA TTTATACAGA	1860
CGTGAGGACA GAAGATTGGA TATTTTCTGC TGTTCACAA GCCCCTGTGT CAGCAGGGTG	1920
ATTCAAGTTG AGCCACAGGC CTACACAGAG CCTCACAGTA ACACCCGGTA CAGCCCCCA	1980
CCCCATACA CCAGCCACAG CTTGCCCCAC GAAACCCATA TCACTATGCA GTCCACCGTT	2040
CAGCTCCGCA CAGAGTATGA CCCTCACAGC CACGTGTACT ACACCACCGC CGAGCCACGC	2100
TCTGAGATCT CTGTACAGCC TGTTACCGTC ACCCAGGACA ACCTCAGCTG TCAGAGTCCC	2160
GAGAGCACCA GCTCTACCAG GGACCTGCTC TCCCAGTTCT CAGACTCCAG CCTCCACTGC	2220
CTCGAGCCCC CCTGCACCA GTGGACACTC TCTTCGTTTG CAGAGAAGCA CTATGCTCCT	2280
TTCTCCTGA AACCCTAAGC CAAGGTTGTG GTAATCCTTC TTTTCCTGGG CTGCTGGGG	2340
GTCAGCCTTT ATGGGACCAC CCGAGTGAGA GACGGGCTGG ACCTCACGGA CATTGTTCCC	2400
CGGGAAACCA GAGAATATGA CTTCATAGCT GCCCAGTTCA AGTACTTCTC TTTCTACAAC	2460
ATGTATATAG TCACCCAGAA AGCAGACTAC CCGAATATCC AGCACCTACT TTACGACCTT	2520
CATAAGAGTT TCAGCAATGT GAAGTATGTC ATGCTGGAGG AGAACAAGCA ACTTCCCCAA	2580
ATGTGGCTGC ACTACTTTAG AGACTGGCTT CAAGGACTTC AGGATGCATT TGACAGTGAC	2640
TGGGAAACTG GGAGGATCAT GCCAAACAAT TATAAAAATG GATCAGATGA CGGGGTCTC	2700
GCTTACAAAC TCCTGGTGCA GACTGGCAGC CGAGACAAGC CCATCGACAT TAGTCAGTTG	2760
ACTAAACAGC GTCTGGTAGA CGCAGATGGC ATCATTAAATC CGAGCGCTTT CTACATCTAC	2820
CTGACCGCTT GGGTCAGCAA CGACCCTGTA GCTTACGCTG CCTCCCAGGC CAACATCCGG	2880

CCTCACC CGC CCGAGTGGGT CCATGACAAA GCCGACTACA TGCCAGAGAC CAGGCTGAGA	2940
ATCCCAGCAG CAGAGCCCAT CGAGTACGCT CAGTTCCCTT TCTACCTCAA CGGCCTACGA	3000
GACACCTCAG ACTTTGTGGA AGCCATAGAA AAAGTGAGAG TCATCTGTAA CAACTATACG	3060
AGCCTGGGAC TGTCCAGCTA CCCCAATGGC TACCCCTTCC TGTTCCTGGGA GCAATACATC	3120
AGCCTGCGCC ACTGGCTGCT GCTATCCATC AGCGTGGTGC TGGCCTGCAC GTTCTAGTG	3180
TGCGCAGTCT TCCTCCTGAA CCCCTGGACG GCCGGGATCA TTGTCATGGT CCTGGCTCTG	3240
ATGACCGTTG AGCTCTTTGG CATGATGGGC CTCATTGGGA TCAAGCTGAG TGCTGTGCCT	3300
GTGGTCATCC TGATTGCATC TGTGGCATC GGAGTGGAGT TCACCGTCCA CGTGGCTTTG	3360
GCCTTTCTGA CAGCCATTGG GGACAAGAAC CACAGGGCTA TGCTCGCTCT GGAACACATG	3420
TTTGCTCCCG TTCTGGACGG TGCTGTGTCC ACTCTGCTGG GTGTACTGAT GCTTGCAGGG	3480
TCCGAATTTG ATTTCAATTGT CAGATACTTC TTTGCCGTCC TGGCCATTCT CACCGTCTTG	3540
GGGGTTCTCA ATGGACTGGT TCTGCTGCCT GTCCTCTTAT CCTTCTTTGG ACCGTGTCCT	3600
GAGGTGTCTC CAGCCAATGG CCTAAACCGA CTGCCACTC CTTCGCCTGA GCCGCCTCCA	3660
AGTGTGCTCC GGTTCGCGT GCCTCCTGGT CACACGAACA ATGGGTCTGA TTCCTCCGAC	3720
TCGGAGTACA GCTCTCAGAC CACGGTGTCT GGCATCAGTG AGGAGCTCAG GCAATACGAA	3780
GCACAGCAGG GTGCCGGAGG CCCTGCCCAC CAAGTGATTG TGGAAGCCAC AGAAAACCCT	3840
GTCTTTGCCC GGTCCACTGT GGTCCATCCG GACTCCAGAC ATCAGCCTCC CTTGACCCCT	3900
CGGCAACAGC CCCACCTGGA CTCTGGCTCC TTGTCCCCTG GACGGCAAGG CCAGCAGCCT	3960
CGAAGGGATC CCCCTAGAGA AGGCTTGCGG CCACCCCCCT ACAGACCGCG CAGAGACGCT	4020
TTTGAAATTT CTA CTGAAGG GCATTCTGGC CCTAGCAATA GGGACCGCTC AGGGCCCCGT	4080
GGGGCCCCGT CTCACAACCC TCGGAACCCA ACGTCCACCG CCATGGGCAG CTCTGTGCCC	4140
AGCTACTGCC AGCCCATCAC CACTGTGACG GCTTCTGCTT CGGTGACTGT TGCTGTGCAT	4200
CCCCCGCCTG GACCTGGGCG CAACCCCGA GGGGGGCCCT GTCCAGGCTA TGAGAGCTAC	4260
CCTGAGACTG ATCAGCGGGT ATTTGAGGAT CCTCATGTGC CTTTTCATGT CAGGTGTGAG	4320
AGGAGGGACT CAAAGGTGGA GGT CATAGAG CTACAGGACG TGGAATGTGA GGAGAGGCCG	4380
TGGGGGAGCA GCTCCAATG AGGGTAATTA AAATCTGAAG CAAAGAGGCC AAAGATTGGA	4440
AAGCCCCGCC CCCACCTCTT TCCAGAACTG CTTGAAGAGA ACTGCTTGA ATTATGGGAA	4500
GGCAGTTCAT TGTTACTGTA ACTGATTGTA TTATTKGTG AAATATTTCT ATAAATATTT	4560
AARAGGTGTA CACATGTAAT ATACATGGAA ATGCTGTACA GTCTATTTCC TGGGGCCTCT	4620

CCACTCCTGC CCCAGAGTGG GGAGACCACA GGGGCCCTTT CCCCTGTGTA CATTGGTCTC 4680
 TGTGCCACAA CCAAGCTTAA CTTAGTTTTA AAAAAAATCT CCCAGCATAT GTCGCTGCTG 4740
 CTTAAATATT GTATAATTTA CTTGTATAAT TCTATGCAA TATTGCTTAT GTAATAGGAT 4800
 TATTTGTAAA GGTTCCTGTT TAAAATATTT TAAATTTGCA TATCACAACC CTGTGGTAGG 4860
 ATGAATTGTT ACTGTTAAC TTTGAACACG CTATGCGTGG TAATTGTTA ACGAGCAGAC 4920
 ATGAAGAAAA CAGGTTAATC CCACTGGCTT CTCTAGGGGT AGTTGTATAT GGTTGCGATG 4980
 GGTGGATGTG TGTGTGCATG TGACTTTCCA ATGTACTGTA TTGTGGTTTG TTGTTGTTGT 5040
 TGCTGTTGTT GTTCATTTTG GTGTTTTTGG TTGCTTTGTA TGATCTTAGC TCTGGCCTAG 5100
 GTGGGCTGGG AAGGTCCAGG TCTTTTTCTG TCGTGATGCT GGTGGAAGG TGACCCCAAT 5160
 CATCTGTCCT ATTCTCTGGG ACTATTC 5187

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1434 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ala Ser Ala Gly Asn Ala Ala Gly Ala Leu Gly Arg Gln Ala Gly
 1 5 10 15
 Gly Gly Arg Arg Arg Arg Thr Gly Gly Pro His Arg Ala Ala Pro Asp
 20 25 30
 Arg Asp Tyr Leu His Arg Pro Ser Tyr Cys Asp Ala Ala Phe Ala Leu
 35 40 45
 Glu Gln Ile Ser Lys Gly Lys Ala Thr Gly Arg Lys Ala Pro Leu Trp
 50 55 60
 Leu Arg Ala Lys Phe Gln Arg Leu Leu Phe Lys Leu Gly Cys Tyr Ile
 65 70 75 80
 Gln Lys Asn Cys Gly Lys Phe Leu Val Val Gly Leu Leu Ile Phe Gly
 85 90 95
 Ala Phe Ala Val Gly Leu Lys Ala Ala Asn Leu Glu Thr Asn Val Glu
 100 105 110
 Glu Leu Trp Val Glu Val Gly Gly Arg Val Ser Arg Glu Leu Asn Tyr
 115 120 125

Thr Arg Gln Lys Ile Gly Glu Glu Ala Met Phe Asn Pro Gln Leu Met
 130 135 140
 Ile Gln Thr Pro Lys Glu Glu Gly Ala Asn Val Leu Thr Thr Glu Ala
 145 150 155 160
 Leu Leu Gln His Leu Asp Ser Ala Leu Gln Ala Ser Arg Val His Val
 165 170 175
 Tyr Met Tyr Asn Arg Gln Trp Lys Leu Glu His Leu Cys Tyr Lys Ser
 180 185 190
 Gly Glu Leu Ile Thr Glu Thr Gly Tyr Met Asp Gln Ile Ile Glu Tyr
 195 200 205
 Leu Tyr Pro Cys Leu Ile Ile Thr Pro Leu Asp Cys Phe Trp Glu Gly
 210 215 220
 Ala Lys Leu Gln Ser Gly Thr Ala Tyr Leu Leu Gly Lys Pro Pro Leu
 225 230 235 240
 Arg Trp Thr Asn Phe Asp Pro Leu Glu Phe Leu Glu Glu Leu Lys Lys
 245 250 255
 Ile Asn Tyr Gln Val Asp Ser Trp Glu Glu Met Leu Asn Lys Ala Glu
 260 265 270
 Val Gly His Gly Tyr Met Asp Arg Pro Cys Leu Asn Pro Ala Asp Pro
 275 280 285
 Asp Cys Pro Ala Thr Ala Pro Asn Lys Asn Ser Thr Lys Pro Leu Asp
 290 295 300
 Val Ala Leu Val Leu Asn Gly Gly Cys Gln Gly Leu Ser Arg Lys Tyr
 305 310 315 320
 Met His Trp Gln Glu Glu Leu Ile Val Gly Gly Thr Val Lys Asn Ala
 325 330 335
 Thr Gly Lys Leu Val Ser Ala His Ala Leu Gln Thr Met Phe Gln Leu
 340 345 350
 Met Thr Pro Lys Gln Met Tyr Glu His Phe Arg Gly Tyr Asp Tyr Val
 355 360 365
 Ser His Ile Asn Trp Asn Glu Asp Arg Ala Ala Ala Ile Leu Glu Ala
 370 375 380
 Trp Gln Arg Thr Tyr Val Glu Val Val His Gln Ser Val Ala Pro Asn
 385 390 395 400
 Ser Thr Gln Lys Val Leu Pro Phe Thr Thr Thr Thr Leu Asp Asp Ile
 405 410 415
 Leu Lys Ser Phe Ser Asp Val Ser Val Ile Arg Val Ala Ser Gly Tyr
 420 425 430
 Leu Leu Met Leu Ala Tyr Ala Cys Leu Thr Met Leu Arg Trp Asp Cys

435	440	445
Ser Lys Ser Gln Gly Ala Val Gly Leu Ala Gly Val Leu Leu Val Ala 450	455	460
Leu Ser Val Ala Ala Gly Leu Gly Leu Cys Ser Leu Ile Gly Ile Ser 465	470	475 480
Phe Asn Ala Ala Thr Thr Gln Val Leu Pro Phe Leu Ala Leu Gly Val 485	490	495
Gly Val Asp Asp Val Phe Leu Leu Ala His Ala Phe Ser Glu Thr Gly 500	505	510
Gln Asn Lys Arg Ile Pro Phe Glu Asp Arg Thr Gly Glu Cys Leu Lys 515	520	525
Arg Thr Gly Ala Ser Val Ala Leu Thr Ser Ile Ser Asn Val Thr Ala 530	535	540
Phe Phe Met Ala Ala Leu Ile Pro Ile Pro Ala Leu Arg Ala Phe Ser 545	550	555 560
Leu Gln Ala Ala Val Val Val Val Phe Asn Phe Ala Met Val Leu Leu 565	570	575
Ile Phe Pro Ala Ile Leu Ser Met Asp Leu Tyr Arg Arg Glu Asp Arg 580	585	590
Arg Leu Asp Ile Phe Cys Cys Phe Thr Ser Pro Cys Val Ser Arg Val 595	600	605
Ile Gln Val Glu Pro Gln Ala Tyr Thr Glu Pro His Ser Asn Thr Arg 610	615	620
Tyr Ser Pro Pro Pro Tyr Thr Ser His Ser Phe Ala His Glu Thr 625	630	635 640
His Ile Thr Met Gln Ser Thr Val Gln Leu Arg Thr Glu Tyr Asp Pro 645	650	655
His Thr His Val Tyr Tyr Thr Thr Ala Glu Pro Arg Ser Glu Ile Ser 660	665	670
Val Gln Pro Val Thr Val Thr Gln Asp Asn Leu Ser Cys Gln Ser Pro 675	680	685
Glu Ser Thr Ser Ser Thr Arg Asp Leu Leu Ser Gln Phe Ser Asp Ser 690	695	700
Ser Leu His Cys Leu Glu Pro Pro Cys Thr Lys Trp Thr Leu Ser Ser 705	710	715 720
Phe Ala Glu Lys His Tyr Ala Pro Phe Leu Leu Lys Pro Lys Ala Lys 725	730	735
Val Val Val Ile Leu Leu Phe Leu Gly Leu Leu Gly Val Ser Leu Tyr 740	745	750

Gly Thr Thr Arg Val Arg Asp Gly Leu Asp Leu Thr Asp Ile Val Pro
 755 760 765
 Arg Glu Thr Arg Glu Tyr Asp Phe Ile Ala Ala Gln Phe Lys Tyr Phe
 770 775 780
 Ser Phe Tyr Asn Met Tyr Ile Val Thr Gln Lys Ala Asp Tyr Pro Asn
 785 790 795 800
 Ile Gln His Leu Leu Tyr Asp Leu His Lys Ser Phe Ser Asn Val Lys
 805 810 815
 Tyr Val Met Leu Glu Glu Asn Lys Gln Leu Pro Gln Met Trp Leu His
 820 825 830
 Tyr Phe Arg Asp Trp Leu Gln Gly Leu Gln Asp Ala Phe Asp Ser Asp
 835 840 845
 Trp Glu Thr Gly Arg Ile Met Pro Asn Asn Tyr Lys Asn Gly Ser Asp
 850 855 860
 Asp Gly Val Leu Ala Tyr Lys Leu Leu Val Gln Thr Gly Ser Arg Asp
 865 870 875 880
 Lys Pro Ile Asp Ile Ser Gln Leu Thr Lys Gln Arg Leu Val Asp Ala
 885 890 895
 Asp Gly Ile Ile Asn Pro Ser Ala Phe Tyr Ile Tyr Leu Thr Ala Trp
 900 905 910
 Val Ser Asn Asp Pro Val Ala Tyr Ala Ala Ser Gln Ala Asn Ile Arg
 915 920 925
 Pro His Arg Pro Glu Trp Val His Asp Lys Ala Asp Tyr Met Pro Glu
 930 935 940
 Thr Arg Leu Arg Ile Pro Ala Ala Glu Pro Ile Glu Tyr Ala Gln Phe
 945 950 955 960
 Pro Phe Tyr Leu Asn Gly Leu Arg Asp Thr Ser Asp Phe Val Glu Ala
 965 970 975
 Ile Glu Lys Val Arg Val Ile Cys Asn Asn Tyr Thr Ser Leu Gly Leu
 980 985 990
 Ser Ser Tyr Pro Asn Gly Tyr Pro Phe Leu Phe Trp Glu Gln Tyr Ile
 995 1000 1005
 Ser Leu Arg His Trp Leu Leu Leu Ser Ile Ser Val Val Leu Ala Cys
 1010 1015 1020
 Thr Phe Leu Val Cys Ala Val Phe Leu Leu Asn Pro Trp Thr Ala Gly
 1025 1030 1035 1040
 Ile Ile Val Met Val Leu Ala Leu M t Thr Val Glu Leu Phe Gly Met
 1045 1050 1055
 Met Gly Leu Ile Gly Ile Lys Leu Ser Ala Val Pro Val Val Ile Leu

1060	1065	1070
Ile Ala Ser Val Gly Ile Gly Val Glu Phe Thr Val His Val Ala Leu 1075	1080	1085
Ala Phe Leu Thr Ala Ile Gly Asp Lys Asn His Arg Ala Met Leu Ala 1090	1095	1100
Leu Glu His Met Phe Ala Pro Val Leu Asp Gly Ala Val Ser Thr Leu 1105	1110	1115 1120
Leu Gly Val Leu Met Leu Ala Gly Ser Glu Phe Asp Phe Ile Val Arg 1125	1130	1135
Tyr Phe Phe Ala Val Leu Ala Ile Leu Thr Val Leu Gly Val Leu Asn 1140	1145	1150
Gly Leu Val Leu Leu Pro Val Leu Leu Ser Phe Phe Gly Pro Cys Pro 1155	1160	1165
Glu Val Ser Pro Ala Asn Gly Leu Asn Arg Leu Pro Thr Pro Ser Pro 1170	1175	1180
Glu Pro Pro Pro Ser Val Val Arg Phe Ala Val Pro Pro Gly His Thr 1185	1190	1195 1200
Asn Asn Gly Ser Asp Ser Ser Asp Ser Glu Tyr Ser Ser Gln Thr Thr 1205	1210	1215
Val Ser Gly Ile Ser Glu Glu Leu Arg Gln Tyr Glu Ala Gln Gln Gly 1220	1225	1230
Ala Gly Gly Pro Ala His Gln Val Ile Val Glu Ala Thr Glu Asn Pro 1235	1240	1245
Val Phe Ala Arg Ser Thr Val Val His Pro Asp Ser Arg His Gln Pro 1250	1255	1260
Pro Leu Thr Pro Arg Gln Gln Pro His Leu Asp Ser Gly Ser Leu Ser 1265	1270	1275 1280
Pro Gly Arg Gln Gly Gln Gln Pro Arg Arg Asp Pro Pro Arg Glu Gly 1285	1290	1295
Leu Arg Pro Pro Pro Tyr Arg Pro Arg Arg Asp Ala Phe Glu Ile Ser 1300	1305	1310
Thr Glu Gly His Ser Gly Pro Ser Asn Arg Asp Arg Ser Gly Pro Arg 1315	1320	1325
Gly Ala Arg Ser His Asn Pro Arg Asn Pro Thr Ser Thr Ala Met Gly 1330	1335	1340
Ser Ser Val Pro Ser Tyr Cys Gln Pro Ile Thr Thr Val Thr Ala Ser 1345	1350	1355 1360
Ala Ser Val Thr Val Ala Val His Pro Pro Pro Gly Pro Gly Arg Asn 1365	1370	1375

Pro Arg Gly Gly Pro Cys Pro Gly Tyr Glu Ser Tyr Pro Glu Thr Asp
1380 1385 1390

His Gly Val Phe Glu Asp Pro His Val Pro Phe His Val Arg Cys Glu
1395 1400 1405

Arg Arg Asp Ser Lys Val Glu Val Ile Glu Leu Gln Asp Val Glu Cys
1410 1415 1420

Glu Glu Arg Pro Trp Gly Ser Ser Ser Asn
1425 1430

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Ile Ile Thr Pro Leu Asp Cys Phe Trp Glu Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Leu Ile Val Gly Gly
1 5

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Pro Phe Phe Trp Glu Gln Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GGACGAATTC AARGTNCAYC ARYTNTGG

28

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGACGAATTC CYTCCCARAA RCANTC

26

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGACGAATTC YTNAGANTGYT TYTGGA

27

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

- (A) DESCRIPTION: /desc = "primer"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CATACCAGCC AAGCTTGTCN GGCCARTGCA T

31

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5288 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GAATTCCGGG GACCGCAAGG AGTGCCGCGG AAGCGCCCGA AGGACAGGCT CGCTCGGCGC	60
CCCGGCTCTC GCTCTTCGCG GAACTGGATG TGGGCAGCGG CGGCCGCGA GACCTCGGGA	120
CCCCCGCGCA ATGTGGCAAT GGAAGGCGCA GGGTCTGACT CCCCAGGAGC GGCCGCGGCC	180
GCAGCGGCAG CAGCGCCGCG CGTGTGAGCA GCAGCAGCGG CTGGTCTGTC AACCGGAGCC	240
CGAGCCCGAG CAGCCTGCGG CCAGCAGCGT CCTCGCAAGC CGAGCGCCCA GGCGCGCCAG	300
GAGCCCGCAG CAGCGGCAGC AGCGCGCGG GCCGCCCGG AAGCCTCCGT CCCCGCGGCG	360
GCGGCGGCGG CGGCGGCGGC AACATGGCCT CGGCTGGTAA CGCCGCCGAG CCCAGGACC	420
GCGGCGGCGG CGGCAGCGGC TGTATCGGTG CCCCAGGAGC GCCGGCTGGA GGCGGGAGGC	480
GCAGACGGAC GGGGGGGCTG CGCCGTGCTG CCGCGCCGGA CCGGGACTAT CTGCACCGGC	540
CCAGCTACTG CGACGCGGCC TTGCTCTGG AGCAGATTTT CAAGGGGAAG GCTACTGGCC	600
GGAAAGCGCC ACTGTGGCTG AGAGCGAAGT TTCAGAGACT CTTATTTAAA CTGGGTGTGT	660
ACATTCAAAA AACTGCGGC AAGTTCTTGG TTGTGGGCCT CCTCATATTT GGGGCCTTCG	720

CGGTGGGATT AAAAGCAGCG AACCTCGAGA CCAACGTGGA GGAGCTGTGG GTGGAAGTTG	780
GAGGACGAGT AAGTCGTGAA TTAAATTATA CTCGCCAGAA GATTGGAGAA GAGGCTATGT	840
TTAATCCTCA ACTCATGATA CAGACCCCTA AAGAAGAAGG TGCTAATGTC CTGACCACAG	900
AAGCGCTCCT ACAACACCTG GACTCGGCAC TCCAGGCCAG CCGTGTCCAT GTATACATGT	960
ACAACAGGCA CTGGAAATTG GAACATTTGT GTTACAAATC AGGAGAGCTT ATCACAGAAA	1020
CAGGTTACAT GGATCAGATA ATAGAATATC TTTACCCTTG TTTGATTATT ACACCTTTGG	1080
ACTGCTTCTG GGAAGGGGCG AAATTACAGT CTGGGACAGC ATACCTCCTA GGTAAACCTC	1140
CTTTGCGGTG GACAAACTTC GACCCTTTGG AATTCCTGGA AGAGTTAAAG AAAATAAACT	1200
ATCAAGTGGA CAGCTGGGAG GAAATGCTGA ATAAGGCTGA GGTTGGTCAT GGTACATGG	1260
ACCGCCCCCTG CCTCAATCCG GCCGATCCAG ACTGCCCCGC CACAGCCCCC AACAAAAATT	1320
CAACCAAACC TCTTGATATG GCCCTTGTTT TGAATGGTGG ATGTCATGGC TTATCCAGAA	1380
AGTATATGCA CTGGCAGGAG GAGTTGATTG TGGGTGGCAC AGTCAAGAAC AGCACTGGAA	1440
AACTCGTCAG CGCCCATGCC CTGCAGACCA TGTTCCAGTT AATGACTCCC AAGCAAATGT	1500
ACGAGCACTT CAAGGGGTAC GAGTATGTCT CACACATCAA CTGGAACGAG GACAAAGCGG	1560
CAGCCATCCT GGAGGCCTGG CAGAGGACAT ATGTGGAGGT GGTTTCATCAG AGTGTCGCAC	1620
AGAACTCCAC TCAAAGGTG CTTTCCTTCA CCACCACGAC CCTGGACGAC ATCCTGAAAT	1680
CCTTCTCTGA CGTCAGTGTG ATCCGCGTGG CCAGCGGCTA CTTACTCATG CTCGCCTATG	1740
CCTGTCTAAC CATGCTGCGC TGGGACTGCT CCAAGTCCCA GGGTGCCGTG GGGCTGGCTG	1800
GCGTCTTGCT GGTTCGACTG TCAGTGGCTG CAGGACTGGG CCTGTGCTCA TTGATCGGAA	1860
TTTCCTTTAA CGCTGCAACA ACTCAGGTTT TGCCATTTCT CGCTCTTGGT GTTGGTGTGG	1920
ATGATGTTTT TCTTCTGGCC CACGCCTTCA GTGAAACAGG ACAGAATAAA AGAATCCCTT	1980
TTGAGGACAG GACCGGGGAG TGCCTGAAGC GCACAGGAGC CAGCGTGGCC CTCACGTCCA	2040
TCAGCAATGT CACAGCCTTC TTCATGGCCG CGTTAATCCC AATCCCGCT CTGCGGGCGT	2100
TCTCCCTCCA GGCAGCGGTA GTAGTGGTGT TCAATTTTGC CATGGTTCGT CTCATTTTTC	2160
CTGCAATTCT CAGCATGGAT TTATATCGAC GCGAGGACAG GAGACTGGAT ATTTTCTGCT	2220
GTTTTACAAG CCCCTGCGTC AGCAGAGTGA TTCAGGTTGA ACCTCAGGCC TACACCGACA	2280
CACACGACAA TACCCGCTAC AGCCCCCAC CTCCCTACAG CAGCCACAGC TTTGCCCATG	2340
AAACGCAGAT TACCATGCAG TCCACTGTCC AGCTCCGCAC GGAGTACGAC CCCCACACGC	2400
ACGTGTACTA CACCACCGCT GAGCCGCGCT CCGAGATCTC TGTGCAGCCC GTCACCGTGA	2460

CACAGGACAC CCTCAGCTGC CAGAGCCCAG AGAGCACCAG CTCCACAAGG GACCTGCTCT	2520
CCCAGTTCTC CGACTCCAGC CTCCACTGCC TCGAGCCCCC CTGTACGAAG TGGACACTCT	2580
CATCTTTTGC TGAGAAGCAC TATGCTCCTT TCCTCTTGAA ACCAAAAGCC AAGGTAGTGG	2640
TGATCTTCCT TTTTCTGGGC TTGCTGGGGG TCAGCCTTTA TGGCACCACC CGAGTGAGAG	2700
ACGGGCTGGA CCTTACGGAC ATTGTACCTC GGGAAACCAG AGAATATGAC TTTATTGCTG	2760
CACAATTCAA ATACTTTTCT TTCTACAACA TGTATATAGT CACCCAGAAA GCAGACTACC	2820
CGAATATCCA GCACTTACTT TACGACCTAC ACAGGAGTTT CAGTAACGTG AAGTATGTCA	2880
TGTTGGAAGA AAACAAACAG CTTCCCAAAA TGTGGCTGCA CTACTTCAGA GACTGGCTTC	2940
AGGGACTTCA GGATGCATTT GACAGTGA CTGAGGAAACCGG GAAAATCATG CCAAACAATT	3000
ACAAGAATGG ATCAGACGAT GGAGTCCTTG CCTACAACT CCTGGTGCAA ACCGGCAGCC	3060
GCGATAAGCC CATCGACATC AGCCAGTTGA CTAAACAGCG TCTGGTGGAT GCAGATGGCA	3120
TCATTAATCC CAGCGCTTTC TACATCTACC TGACGGCTTG GGTGAGCAAC GACCCCGTCG	3180
CGTATGCTGC CTCCAGGCC AACATCCGGC CACACCGACC AGAATGGGTC CACGACAAAG	3240
CCGACTACAT GCCTGAAACA AGGCTGAGAA TCCCGGCAGC AGAGCCCATC GAGTATGCCC	3300
AGTTCCCTTT CTACCTCAAC GGGTTGCGGG ACACCTCAGA CTTGTGGAG GCAATTGAAA	3360
AAGTAAGGAC CATCTGCAGC AACTATACGA GCCTGGGGCT GTCCAGTTAC CCCAACGGCT	3420
ACCCCTTCCT CTTCTGGGAG CAGTACATCG GCCTCCGCCA CTGGCTGCTG CTGTTTCATCA	3480
GCGTGGTGTT GGCCTGCACA TTCCTCGTGT GCGCTGTCTT CTTCTGAAC CCCTGGACGG	3540
CCGGGATCAT TGTGATGGTC CTGGCGCTGA TGACGGTGA GCTGTTCCGC ATGATGGGCC	3600
TCATCGGAAT CAAGCTCAGT GCCGTGCCCC TGGTCATCCT GATCGCTTCT GTTGGCATAG	3660
GAGTGGAGTT CACCGTTCAC GTTGCTTTGG CCTTTCTGAC GGCCATCGGC GACAAGAACC	3720
GCAGGGCTGT GCTTGCCCTG GAGCACATGT TTGCACCCGT CCTGGATGGC GCCGTGTCCA	3780
CTCTGCTGGG AGTGCTGATG CTGGCGGGAT CTGAGTTGGA CTTTATTGTC AGGTATTCT	3840
TTGCTGTGCT GGCATCCTC ACCATCCTCG GCGTTCTCAA TGGGCTGGTT TTGCTTCCCG	3900
TGCTTTTGTG TTTCTTTGGA CCATATCCTG AGGTGTCTCC AGCCAAOCCG TTGAACCGCC	3960
TGCCCCACCC CTCCCCTGAG CCACCCCCCA GCGTGGTCCG CTTGCGCATG CCGCCCCGCC	4020
ACACGCACAG CGGGTCTGAT TCCTCCGACT CGGAGTATAG TTCCAGACG ACAGTGTGAG	4080
GCCTCAGCGA GGAGCTTCGG CACTACGAGG CCCAGCAGGG CGCGGGAGGC CCTGCCCCACC	4140
AAGTGATCGT GGAAGCCACA GAAAACCCCG TCTTCGCCCA CTCCACTGTG GTCCATCCCG	4200

AATCCAGGCA TCACCCACCC TCGAACCCGA GACAGCAGCC CCACCTGGAC TCAGGGTCCC 4260
 TGCCTCCCGG ACGGCAAGGC CAGCAGCCCC GCAGGGACCC CCCCAGAGAA GGCTTGTGGC 4320
 CACCCCTCTA CAGACCGCGC AGAGACGCTT TTGAAATTTC TACTGAAGGG CATTCTGGCC 4380
 CTAGCAATAG GGCCCGCTGG GGCCCTCGCG GGGCCCGTTC TCACAACCCT CGGAACCCAG 4440
 CGTCCACTGC CATGGGCAGC TCCGTGCCCC GCTACTGCCA GCCCATCACC ACTGTGACGG 4500
 CTTCTGCCTC CGTGA CTGTC GCCGTGCACC CGCCGCTGT CCCTGGGCCT GGGCGGAACC 4560
 CCCGAGGGGG ACTCTGCCCA GGCTACCCTG AGACTGACCA CGGCCTGTTT GAGGACCCCC 4620
 ACGTGCCTTT CCACGTCCGG TGTGAGAGGA GGGATTGAA GGTGGAAGTC ATTGAGCTGC 4680
 AGGACGTGGA ATGCGAGGAG AGGCCCCGGG GAAGCAGCTC CAACTGAGGG TGATTAAAT 4740
 CTGAAGCAA GAGGCCAAAG ATTGGAACC CCCCACCCCC ACCTCTTCC AGAACTGCTT 4800
 GAAGAGAACT GGTGGAGTT ATGGAAAAGA TGCCCTGTGC CAGGACAGCA GTTCATTGTT 4860
 ACTGTAACCG ATTGTATTAT TTGTATAAT ATTTCTATAA ATATTTAAGA GATGTACACA 4920
 TGTGTAATAT AGGAAGGAAG GATGTAAAGT GGTATGATCT GGGGCTTCTC CACTCCTGCC 4980
 CCAGAGTGTG GAGGCCACAG TGGGGCCTCT CCGTATTGT GCATTGGGCT CCGTGCCACA 5040
 ACCAAGCTTC ATTAGCTTA AATTCAGCA TATGTTGCTG CTGCTTAAAT ATTGTATAAT 5100
 TTA CTGTAT AATTCTATGC AAATATTGCT TATGTAATAG GATTATTTG TAAAGGTTTC 5160
 TGTTTAAAAT ATTTTAAAT TGCATATCAC AACCTGTGG TAGTATGAAA TGTTACTGTT 5220
 AACTTCAAA CACGCTATGC GTGATAATTT TTTGTTTAA TGAGCAGATA TGAAGAAAGC 5280
 CCGGAATT 5288

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1447 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Met Ala Ser Ala Gly Asn Ala Ala Glu Pro Gln Asp Arg Gly Gly Gly
 1 5 10 15
 Gly Ser Gly Cys Ile Gly Ala Pr Gly Arg Pro Ala Gly ly Gly Arg
 20 25 30

Arg Arg Arg Thr Gly Gly Leu Arg Arg Ala Ala Ala Pro Asp Arg Asp
 35 40 45
 Tyr Leu His Arg Pro Ser Tyr Cys Asp Ala Ala Phe Ala Leu Glu Gln
 50 55 60
 Ile Ser Lys Gly Lys Ala Thr Gly Arg Lys Ala Pro Leu Trp Leu Arg
 65 70 75 80
 Ala Lys Phe Gln Arg Leu Leu Phe Lys Leu Gly Cys Tyr Ile Gln Lys
 85 90 95
 Asn Cys Gly Lys Phe Leu Val Val Gly Leu Leu Ile Phe Gly Ala Phe
 100 105 110
 Ala Val Gly Leu Lys Ala Ala Asn Leu Glu Thr Asn Val Glu Glu Leu
 115 120 125
 Trp Val Glu Val Gly Gly Arg Val Ser Arg Glu Leu Asn Tyr Thr Arg
 130 135 140
 Gln Lys Ile Gly Glu Glu Ala Met Phe Asn Pro Gln Leu Met Ile Gln
 145 150 155 160
 Thr Pro Lys Glu Glu Gly Ala Asn Val Leu Thr Thr Glu Ala Leu Leu
 165 170 175
 Gln His Leu Asp Ser Ala Leu Gln Ala Ser Arg Val His Val Tyr Met
 180 185 190
 Tyr Asn Arg Gln Trp Lys Leu Glu His Leu Cys Tyr Lys Ser Gly Glu
 195 200 205
 Leu Ile Thr Glu Thr Gly Tyr Met Asp Gln Ile Ile Glu Tyr Leu Tyr
 210 215 220
 Pro Cys Leu Ile Ile Thr Pro Leu Asp Cys Phe Trp Glu Gly Ala Lys
 225 230 235 240
 Leu Gln Ser Gly Thr Ala Tyr Leu Leu Gly Lys Pro Pro Leu Arg Trp
 245 250 255
 Thr Asn Phe Asp Pro Leu Glu Phe Leu Glu Glu Leu Lys Lys Ile Asn
 260 265 270
 Tyr Gln Val Asp Ser Trp Glu Glu Met Leu Asn Lys Ala Glu Val Gly
 275 280 285
 His Gly Tyr Met Asp Arg Pro Cys Leu Asn Pro Ala Asp Pro Asp Cys
 290 295 300
 Pro Ala Thr Ala Pro Asn Lys Asn Ser Thr Lys Pro Leu Asp Met Ala
 305 310 315 320
 Leu Val Leu Asn Gly Gly Cys His Gly Leu Ser Arg Lys Tyr Met His
 325 330 335
 Trp Gln Glu Glu Leu Ile Val Gly Gly Thr Val Lys Asn Ser Thr Gly

340	345	350
Lys Leu Val Ser Ala His Ala	Leu Gln Thr Met Phe	Gln Leu Met Thr
355	360	365
Pro Lys Gln Met Tyr Glu His	Phe Lys Gly Tyr Glu Tyr Val Ser His	
370	375	380
Ile Asn Trp Asn Glu Asp Lys Ala Ala Ala	Ile Leu Glu Ala Trp Gln	
385	390	395
Arg Thr Tyr Val Glu Val Val His Gln Ser Val Ala Gln Asn Ser Thr		
405	410	415
Gln Lys Val Leu Ser Phe Thr Thr Thr Thr	Leu Asp Asp Ile Leu Lys	
420	425	430
Ser Phe Ser Asp Val Ser Val Ile Arg Val Ala Ser Gly Tyr Leu Leu		
435	440	445
Met Leu Ala Tyr Ala Cys Leu Thr Met Leu Arg Trp Asp Cys Ser Lys		
450	455	460
Ser Gln Gly Ala Val Gly Leu Ala Gly Val Leu Leu Val Ala Leu Ser		
465	470	475
Val Ala Ala Gly Leu Gly Leu Cys Ser Leu Ile Gly Ile Ser Phe Asn		
485	490	495
Ala Ala Thr Thr Gln Val Leu Pro Phe Leu Ala Leu Gly Val Gly Val		
500	505	510
Asp Asp Val Phe Leu Leu Ala His Ala Phe Ser Glu Thr Gly Gln Asn		
515	520	525
Lys Arg Ile Pro Phe Glu Asp Arg Thr Gly Glu Cys Leu Lys Arg Thr		
530	535	540
Gly Ala Ser Val Ala Leu Thr Ser Ile Ser Asn Val Thr Ala Phe Phe		
545	550	555
Met Ala Ala Leu Ile Pro Ile Pro Ala Leu Arg Ala Phe Ser Leu Gln		
565	570	575
Ala Ala Val Val Val Val Phe Asn Phe Ala Met Val Leu Leu Ile Phe		
580	585	590
Pro Ala Ile Leu Ser Met Asp Leu Tyr Arg Arg Glu Asp Arg Arg Leu		
595	600	605
Asp Ile Phe Cys Cys Phe Thr Ser Pro Cys Val Ser Arg Val Ile Gln		
610	615	620
Val Glu Pro Gln Ala Tyr Thr Asp Thr His Asp Asn Thr Arg Tyr Ser		
625	630	635
Pro Pro Pro Pro Tyr Ser Ser His Ser Phe Ala His Glu Thr Gln Il		
645	650	655

Thr M t Gln Ser Thr Val Gln Leu Arg Thr Glu Tyr Asp Pro His Thr
 660 665 670
 His Val Tyr Tyr Thr Thr Ala Glu Pro Arg Ser Glu Ile Ser Val Gln
 675 680 685
 Pro Val Thr Val Thr Gln Asp Thr Leu Ser Cys Gln Ser Pro Glu Ser
 690 695 700
 Thr Ser Ser Thr Arg Asp Leu Leu Ser Gln Phe Ser Asp Ser Ser Leu
 705 710 715 720
 His Cys Leu Glu Pro Pro Cys Thr Lys Trp Thr Leu Ser Ser Phe Ala
 725 730 735
 Glu Lys His Tyr Ala Pro Phe Leu Leu Lys Pro Lys Ala Lys Val Val
 740 745 750
 Val Ile Phe Leu Phe Leu Gly Leu Leu Gly Val Ser Leu Tyr Gly Thr
 755 760 765
 Thr Arg Val Arg Asp Gly Leu Asp Leu Thr Asp Ile Val Pro Arg Glu
 770 775 780
 Thr Arg Glu Tyr Asp Phe Ile Ala Ala Gln Phe Lys Tyr Phe Ser Phe
 785 790 795 800
 Tyr Asn Met Tyr Ile Val Thr Gln Lys Ala Asp Tyr Pro Asn Ile Gln
 805 810 815
 His Leu Leu Tyr Asp Leu His Arg Ser Phe Ser Asn Val Lys Tyr Val
 820 825 830
 Met Leu Glu Glu Asn Lys Gln Leu Pro Lys Met Trp Leu His Tyr Phe
 835 840 845
 Arg Asp Trp Leu Gln Gly Leu Gln Asp Ala Phe Asp Ser Asp Trp Glu
 850 855 860
 Thr Gly Lys Ile Met Pro Asn Asn Tyr Lys Asn Gly Ser Asp Asp Gly
 865 870 875 880
 Val Leu Ala Tyr Lys Leu Leu Val Gln Thr Gly Ser Arg Asp Lys Pro
 885 890 895
 Ile Asp Ile Ser Gln Leu Thr Lys Gln Arg Leu Val Asp Ala Asp Gly
 900 905 910
 Ile Ile Asn Pro Ser Ala Phe Tyr Ile Tyr Leu Thr Ala Trp Val Ser
 915 920 925
 Asn Asp Pro Val Ala Tyr Ala Ala Ser Gln Ala Asn Ile Arg Pro His
 930 935 940
 Arg Pro Glu Trp Val His Asp Lys Ala Asp Tyr Met Pro Glu Thr Arg
 945 950 955 960
 Leu Arg Ile Pro Ala Ala Glu Pr Ile Glu Tyr Ala Gln Phe Pro Phe

965	970	975
Tyr Leu Asn Gly Leu Arg Asp Thr Ser Asp Phe Val Glu Ala Ile Glu 980 985 990		
Lys Val Arg Thr Ile Cys Ser Asn Tyr Thr Ser Leu Gly Leu Ser Ser 995 1000 1005		
Tyr Pro Asn Gly Tyr Pro Phe Leu Phe Trp Glu Gln Tyr Ile Gly Leu 1010 1015 1020		
Arg His Trp Leu Leu Leu Phe Ile Ser Val Val Leu Ala Cys Thr Phe 1025 1030 1035 1040		
Leu Val Cys Ala Val Phe Leu Leu Asn Pro Trp Thr Ala Gly Ile Ile 1045 1050 1055		
Val Met Val Leu Ala Leu Met Thr Val Glu Leu Phe Gly Met Met Gly 1060 1065 1070		
Leu Ile Gly Ile Lys Leu Ser Ala Val Pro Val Val Ile Leu Ile Ala 1075 1080 1085		
Ser Val Gly Ile Gly Val Glu Phe Thr Val His Val Ala Leu Ala Phe 1090 1095 1100		
Leu Thr Ala Ile Gly Asp Lys Asn Arg Arg Ala Val Leu Ala Leu Glu 1105 1110 1115 1120		
His Met Phe Ala Pro Val Leu Asp Gly Ala Val Ser Thr Leu Leu Gly 1125 1130 1135		
Val Leu Met Leu Ala Gly Ser Glu Phe Asp Phe Ile Val Arg Tyr Phe 1140 1145 1150		
Phe Ala Val Leu Ala Ile Leu Thr Ile Leu Gly Val Leu Asn Gly Leu 1155 1160 1165		
Val Leu Leu Pro Val Leu Leu Ser Phe Phe Gly Pro Tyr Pro Glu Val 1170 1175 1180		
Ser Pro Ala Asn Gly Leu Asn Arg Leu Pro Thr Pro Ser Pro Glu Pro 1185 1190 1195 1200		
Pro Pro Ser Val Val Arg Phe Ala Met Pro Pro Gly His Thr His Ser 1205 1210 1215		
Gly Ser Asp Ser Ser Asp Ser Glu Tyr Ser Ser Gln Thr Thr Val Ser 1220 1225 1230		
Gly Leu Ser Glu Glu Leu Arg His Tyr Glu Ala Gln Gln Gly Ala Gly 1235 1240 1245		
Gly Pro Ala His Gln Val Ile Val Glu Ala Thr Glu Asn Pro Val Phe 1250 1255 1260		
Ala His Ser Thr Val Val His Pro Glu Ser Arg His His Pr Pr S r 1265 1270 1275 1280		

Asn Pro Arg Gln Gln Pro His Leu Asp Ser Gly Ser Leu Pro Pro Gly
 1285 1290 1295
 Arg Gln Gly Gln Gln Pro Arg Arg Asp Pro Pro Arg Glu Gly Leu Trp
 1300 1305 1310
 Pro Pro Leu Tyr Arg Pro Arg Arg Asp Ala Phe Glu Ile Ser Thr Glu
 1315 1320 1325
 Gly His Ser Gly Pro Ser Asn Arg Ala Arg Trp Gly Pro Arg Gly Ala
 1330 1335 1340
 Arg Ser His Asn Pro Arg Asn Pro Ala Ser Thr Ala Met Gly Ser Ser
 1345 1350 1355 1360
 Val Pro Gly Tyr Cys Gln Pro Ile Thr Thr Val Thr Ala Ser Ala Ser
 1365 1370 1375
 Val Thr Val Ala Val His Pro Pro Pro Val Pro Gly Pro Gly Arg Asn
 1380 1385 1390
 Pro Arg Gly Gly Leu Cys Pro Gly Tyr Pro Glu Thr Asp His Gly Leu
 1395 1400 1405
 Phe Glu Asp Pro His Val Pro Phe His Val Arg Cys Glu Arg Arg Asp
 1410 1415 1420
 Ser Lys Val Glu Val Ile Glu Leu Gln Asp Val Glu Cys Glu Glu Arg
 1425 1430 1435 1440
 Pro Arg Gly Ser Ser Ser Asn
 1445

WHAT IS CLAIMED IS:

1. A DNA sequence other than present in a chromosome encoding a *patched* gene other than the *Drosophila patched* gene or fragment thereof of at least about 12bp
5 different from the sequence of the *Drosophila patched* gene.
2. A DNA sequence according to Claim 1, wherein said *patched* gene is a mammalian gene.
- 10 3. A DNA sequence according to Claim 1 for human, mouse, mosquito, butterfly or beetle *patched* gene.
4. A DNA sequence according to Claim 3, wherein said DNA sequence is a human sequence.
- 15 5. A DNA sequence according to Claim 4, wherein said DNA sequence is a mouse sequence.
6. A DNA sequence according to Claim 1, wherein said DNA sequence is a
20 fragment of at least about 18bp.
7. A DNA sequence according to Claim 1 joined to a DNA sequence comprising a restriction enzyme recognition sequence.
- 25 8. An expression cassette comprising a transcriptional initiation region functional in an expression host, a DNA sequence according to Claim 1 under the transcriptional regulation of said transcriptional initiation region, and a transcriptional termination region functional in said expression host.
- 30 9. An expression cassette according to Claim 8, wherein said transcriptional initiation region is heterologous to said DNA sequence according to Claim 1.

10. An expression cassette according to Claim 8, wherein said transcriptional initiation region is homologous to said DNA sequence according to Claim 1 and includes the enhancer region.
- 5 11. A cell comprising an expression cassette according to Claim 8 as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of said expression cassette into said host cell and the cellular progeny of said host cell.
- 10 12. A cell according to Claim 11, further comprising the *patched* protein in the cellular membrane of said cell.
13. A cell according to Claim 11, wherein said *patched* protein is a mouse *patched* protein.
- 15 14. A cell according to Claim 11, wherein said *patched* gene is a human *patched* protein.
15. A cell according to Claim 11, wherein said transcriptional initiation region is a
- 20 *Drosophila patched* gene transcriptional initiation region comprising the promoter and enhancer joined to a heterologous gene.
16. A cell comprising an expression cassette comprising a transcriptional initiation region functional in an expression host, said transcriptional initiation region
- 25 consisting of a 5' non-coding region regulating the transcription of *patched* protein comprising the promoter and enhancer, a marker gene, and a transcriptional termination region, as part of an extrachromosomal element or integrated into the genome of a host cell as a result of introduction of said expression cassette into said host, and the cellular progeny thereof.

30

17. A cell according to Claim 16, wherein said transcriptional initiation region is the *Drosophila* region.
18. A method for following embryonic development employing the *patched* protein in an embryo, said method comprising:
- 5 integrating an expression cassette comprising a transcriptional initiation region functional in embryonic host cells, said transcriptional initiation region consisting of a 5' non-coding region regulating the transcription of *patched* protein, a marker gene, and a transcriptional termination region, wherein said embryonic host cells are
- 10 capable of developing into a fetus;
- growing said embryonic host cells, whereby proliferation and differentiation occur; and
- locating cells comprising expression of the *patched* protein by means of expression of said marker gene.
- 15 19. A method for producing *patched* protein, said method comprising:
- growing a cell according to Claim 11, whereby said *patched* protein is expressed; and
- isolating said *patched* protein free of other proteins.
- 20 20. A method for screening candidate compounds for binding affinity to the *patched* protein, said method comprising:
- combining said candidate protein with a vertebrate or invertebrate cell comprising said *patched* protein in the membrane of said cell and an expression
- 25 cassette comprising a transcriptional initiation region functional in said cell, a DNA sequence according to Claim 1 comprising the entire coding sequence under the transcriptional regulation of said transcriptional initiation region, and a transcriptional termination region functional in said cell, expressing said *patched* protein in said cell; and
- 30 assaying for the binding of said candidate compound to said *patched* protein.

21. A method for screening candidate compounds for agonist activity with the *patched* protein, said method comprising:
- combining said candidate protein with a vertebrate or invertebrate cell comprising said *patched* protein in the membrane of said cell and an expression
- 5 cassette comprising a transcriptional initiation region functional in an expression host, said transcriptional initiation region consisting of a 5' non-coding region regulating the transcription of *patched* protein, a marker gene, and a transcriptional termination region, as part of an extrachromosomal element or integrated into the genome of a host cell; and
- 10 assaying for the expression of said marker gene.
22. A monoclonal antibody binding specifically to a *patched* protein, other than the *Drosophila patched* protein.

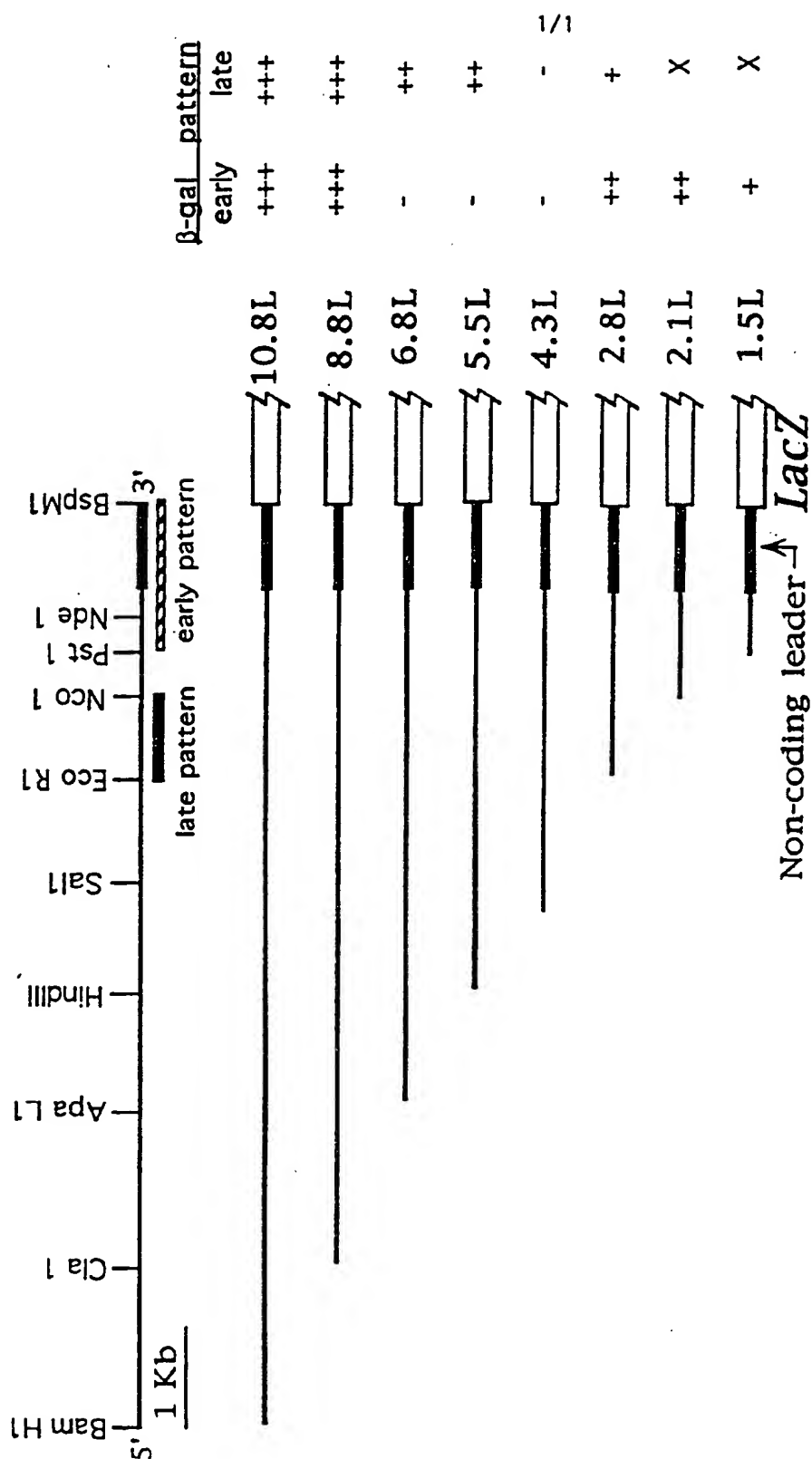


FIGURE 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/13233

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 5/00, 15/00; C07H 21/00
US CL : 536/23.1; 435/69.1, 172.3, 240.2, 320.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1; 435/69.1, 172.3, 240.2, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Nature, Volume 341, issued 12 October 1989, Nakano et al., "A protein with several possible membrane-spanning domains encoded by the Drosophila segment polarity gene patched", pages 508-513, see the entire document.	1-10 and 18
Y	Cell, Volume 59, issued 17 November 1989, Hooper et al., "The Drosophila patched gene encodes a putative membrane protein required for segmental patterning", pages 751-765, see the entire document.	1-10 and 18
Y	Gene, Volume 112, issued 1992, Chavrier et al., "The complexity of the Rab and Rho GTP-binding protein subfamilies revealed by a PCR cloning approach", pages 261-264, see the entire document.	1-10

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A document defining the general state of the art which is not considered to be of particular relevance	*X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*E earlier document published on or after the international filing date	*Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, each combination being obvious to a person skilled in the art
*L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G document member of the same patent family
*O document referring to an oral disclosure, use, exhibition or other means	
*P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

19 JANUARY 1996

Date of mailing of the international search report

31 JAN 1996

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/13233

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Biochemistry, Volume 31, No. 44, issued 1992, Ma et al., "Molecular cloning and characterization of rKlk10, a cDNA encoding T-kininogenase from rat submandibular gland and kidney", pages 10922-10928, see the experimental procedures on page 10923.	1-10
Y	Gene, Volume 74, issued 1988, Thummel et al., "Vectors for Drosophila P-element-mediated transformation and tissue culture transfection", pages 445-456, see the entire document.	8-10 and 18
Y	Developmental Genetics, Volume 12, issued 1991, Perrimon et al., "Generating lineage-specific markers to study Drosophila development", pages 238-252, see the entire document.	8-10 and 18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/13233

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-10 AND 18

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/13233

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, DIALOG

search terms: patched, gene, cloning, PCR, human, mammalian, mouse, mosquito, butterfly, beetle, DNA, drosophila, embryo, gal, galactosidase, develop, review, inventors' names

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-10 and 18, drawn to a DNA sequence encoding a patched gene, an expression cassette, and a method for following embryonic development by integrating the expression cassette.

Group II, claims 11-17 and 19, drawn to a cell and a method for producing patched protein by growing the cell.

Group III, claim 20, drawn to a method for screening candidate compounds for binding affinity to the patched protein.

Group IV, claim 21, drawn to a method for screening candidate compounds for agonist activity with the patched protein.

Group V, claim 22, drawn to a monoclonal antibody specific for a patched protein.

The inventions listed as Groups I-V do not relate to a single inventive concept under PCT Rule 13.1, because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I, II and V are not so linked as to form a single inventive concept because the DNA sequence of Group I, the cell of Group II and the monoclonal antibody of Group V are drawn to three different products.

Groups II and III are not so linked as to form a single inventive concept because they are drawn to materially different methods. The method of Group II involves growing a cell while the method of Group III involves combining a candidate compound with a cell and then assaying for binding.

Groups II and IV are not so linked as to form a single inventive concept because they are drawn to materially different methods. The method of Group II involves growing a cell while the method of Group IV involves combining a candidate compound with a cell and then assaying for expression of a marker gene.